UNIVERSITÀ DEGLI STUDI DI GENOVA

Dipartimento di Neuroscienze, Riabilitazione, Oftalmologia, Genetica e Scienze Materno-Infantili

Dottorato di Ricerca in Scienze Pediatriche – XXXVIII ciclo

Coordinatore: *Ampl.mo Prof. Pasquale Striano*

Proteomic Analysis of Cerebrospinal Fluid and Plasma during Neonatal Late-onset Sepsis Evaluations: Functional Insights into Blood-Cerebrospinal Fluid Barrier Dynamics

Tutor:

Chiar.mo Prof. Luca Antonio Ramenghi

Candidato:

Dr. Francesco Vinci

A.A. 2025/2026

Abstract

Background: Neonatal Late-Onset Sepsis (LOS) is a major contributor to adverse neurodevelopmental outcomes, even in the absence of diagnosed meningitis. The mechanisms linking systemic inflammation to neurological impairment in infants remain unclear. Experimental evidence suggests that central nervous system (CNS) involvement may occur through alteration of brain barrier systems. This study aimed to investigate the neonatal blood-cerebrospinal fluid (CSF) barrier, as a dynamic interface between systemic circulation and CNS, using proteomic profiling to assess its functional behaviour, integrity, and associated biochemical processes during systemic inflammation.

Methods: In this prospective monocentric pilot study conducted in a tertiary Neonatal Intensive Care Unit, temporally matched CSF and plasma samples were collected from term and preterm infants undergoing evaluation for suspected LOS. Proteomic profiling was performed on 45 paired samples using high-resolution liquid chromatography mass spectrometry. Infants were stratified in three groups based on C-reactive protein (CRP) dynamics (not inflamed, evolving inflammation, and overt inflammation), representing increasing systemic inflammatory burden. Differential protein expression was assessed by ANOVA and t-tests, and functional enrichment analyses were conducted to identify modulated pathways.

Results: Significant proteomic modulation was observed, with 57 differentially expressed proteins in CSF and 20 in plasma across CRP dynamics-defined groups. Four proteins - CRP, complement 9 (C9), leucine-rich alpha-2-glycoprotein 1 (LRG1), and alpha-fetoprotein (AFP) - were consistently modulated in both compartments. This pattern supports a model of selective transfer of acute-phase proteins (APPs) from plasma to CSF, indicating regulated blood-CSF barrier permeability rather than overt structural disruption. Functional enrichment analyses revealed negative enrichment of neurogenesis- and axon guidance-related pathways, and positive enrichment of cytoskeleton organization pathways in the CSF proteome with increasing inflammation. Among culture-positive infants, only mannan-binding lectin serine protease 1 (MASP1) was significantly down-regulated in

CSF. Given that MASP1 consumption has been associated with disseminated intravascular coagulation in septic patients, its reduction in CSF may reflect inflammation-related neurovascular-coagulation dynamics.

Conclusion: Systemic inflammation during suspected LOS is associated with marked and compartment-specific proteomic changes in neonates without meningitis. The findings reveal a dual signature consisting of selective APPs transfer across the blood-CSF barrier and modulation of neurodevelopmental and cytoskeletal pathways in CSF. These results support the concept of functional barrier modulation during neonatal inflammation and provide insight into biochemical mechanisms potentially contributing to subtle brain injury. MASP1 modulation further suggests a possible link between inflammatory and neurovascular-coagulation pathways. Identified proteins represent candidate biomarkers of CNS involvement and warrant further validation in larger cohorts.

Keywords

Systemic inflammation, Neonatal late-onset sepsis, Preterm, Neuroinflammation, Sepsis-associated encephalopathy, Blood-CSF barrier, Blood-brain barrier, Proteomics, Mass spectrometry

1. Introduction

1.1 Neonatal Vulnerability and Inflammatory Insults to the Developing Brain

Neonates, particularly those born preterm or with low birth weight (VLBW), are at an increased risk for neurodevelopmental impairments due to their vulnerability to inflammatory insults, especially in the context of late-onset sepsis (LOS). Sepsis in neonates evokes a systemic inflammatory response that can significantly disrupt normal brain development and function, even in the absence of meningitis. [1] Research indicates that the mechanisms underlying neurological involvement during sepsis, such as excessive microglial activation and blood-brain barrier (BBB) dysfunction, are critical contributors to neuroinflammation and subsequent cognitive deficits. It is believed that the BBB, which in normal conditions serves as a protective barrier to regulate nutrient and ion transport, would be possibly involved and compromised during sepsis, allowing pro-inflammatory cytokines and immune cells to infiltrate the central nervous system (CNS). This dysregulation has been associated with neuronal injury and adverse clinical outcomes such as chronic lung disease and neurodevelopmental impairments. [2], [3], [4]

Neonatal neuroinflammation can lead to an array of detrimental effects, including damage to synaptic integrity and alterations in neurogenesis. Previous proteomic analyses on animal and human newborn have revealed that specific proteins associated with neurodevelopment are downregulated in cerebrospinal fluid (CSF) of neonates experiencing inflammatory conditions prompted by infections, signifying a direct impact on synaptic plasticity and overall brain health. [5], [6] This protein depletion aligns with findings that systemic inflammation can activate microglial cells, which, while typically protective, may induce neurotoxic responses under sustained inflammatory conditions. [7], [8] Furthermore, recent studies have indicated that even transient inflammatory responses can have long-lasting effects on brain structure and function, implicating a complex interplay between neuroimmune regulation and developmental outcomes. [9], [10], [11]

The vulnerability of the neonatal brain also extends to specific neurodevelopmental pathways. The developing brain exhibits heightened sensitivity to environmental insults, including inflammation, which can precipitate cognitive dysfunctions later in life. Infections during infancy, characterized by the presence of cytokines like interleukin-6 (IL-6), can significantly impact neurodevelopmental trajectories by promoting inflammatory states that disrupt neural connectivity and alter the execution of neurodevelopmental programs crucial for cognitive and motor function. [12], [13], [14]

Moreover, exploiting the interactions between neuroinflammation and other physiological systems highlights new avenues for therapeutic strategies to mitigate risk factors associated with impaired neurodevelopment in preterm infants. Research focusing on the microglia-leukocyte interaction in the context of cerebral ischemia has revealed that optimizing these responses may enhance recovery and protect against long-term neurodevelopmental impairments associated with neonatal sepsis. [15], [16] As understanding deepens around the proteomic changes and immunological factors involved in these conditions, targeted interventions such as modulating cytokine production or enhancing the integrity of the BBB could emerge as promising approaches. [5], [17]

In conclusion, addressing neonatal vulnerability in the face of inflammatory insults requires a nuanced understanding of the dynamic interplay between the developing brain, systemic inflammation, and the microenvironment provided by the BBB and immune responses. With ongoing advancements in proteomic research and immunological interventions, opportunities for enhancing therapeutic approaches to safeguard neurological outcomes in at-risk neonates are increasingly in focus. Enhanced understanding of these mechanisms not only holds promise for immediate clinical applications but also provides a foundational knowledge base for developing preventative strategies aiming to improve long-term developmental success in this vulnerable population. [18], [19], [20]

1.2 Neonatal Sepsis as a Model of Systemic Inflammation

1.2.1 Definition and Clinical Classification of Neonatal Sepsis

Neonatal sepsis is a significant clinical concern characterized primarily by a systemic inflammatory response to infection occurring in the first 28 days of life. It is categorized into two main forms: early-onset sepsis (EOS) and late-onset sepsis (LOS). Early-onset sepsis typically manifests within the first 72 hours of life, often resulting from vertical transmission of pathogens from the mother during labor, notably Group B *Streptococcus* and *E. coli*. Conversely, LOS occurs after 72 hours of life, usually stemming from hospital-acquired infections linked to invasive procedures or compromised skin barriers in preterm neonates. The distinction between EOS and LOS is clinically vital as it influences treatment protocols and prognostic assessments. [21], [22], [23]

The clinical classification of neonatal sepsis is augmented by standardized diagnostic criteria encompassing both clinical and laboratory parameters. Key clinical criteria may include lethargy, poor feeding, temperature instability, and respiratory distress, which need to be interpreted in the context of associated laboratory findings such as elevated C-reactive protein (CRP) levels and abnormal white blood cell counts. [4], [21], [22] While these clinical and laboratory indicators are pivotal for the early identification of sepsis, challenges in relying solely on microbiological definitions persist. Definitive diagnosis often necessitates isolating the causative organism; however, cultures may remain negative in a substantial percentage of cases, leading to a clinical paradox where infants with severe illness may not be accurately diagnosed. Factors such as prior antibiotic therapy prior to cultures can further obscure definitive microbiological identification, necessitating a reliance on clinical judgment and complementary diagnostic assessments. [18], [24]

Additionally, the limitations of microbiological definitions draw attention to the importance of understanding the broader spectrum of neonatal sepsis. Recent advances in non-culture-based diagnostic techniques, such as proteomics and metabolomics, have emerged, offering potential insights into the host immune response and pathogen. [25], [26] Such innovations may enhance early

diagnosis and treatment strategies, reducing reliance on traditional microbiological methodologies that fail to capture the dynamic inflammatory processes in neonates suffering from sepsis.

1.2.2 Inflammatory Response Syndrome in the Newborn – Role of C-Reactive Protein

The systemic inflammatory response syndrome (SIRS) in the newborn context represents a complex interplay of inflammatory mediators initiated in response to infection. This response is often assessed through several biomarkers, with CRP being one of the most extensively studied indicators of systemic inflammation. CRP is an acute-phase protein synthesized by the liver, and its levels rise significantly in response to inflammation, infection, and tissue injury. This rise typically occurs within 6 to 12 hours following the inflammatory insult. Measuring CRP levels has significant clinical relevance, as elevated values can assist in confirming or ruling out the diagnosis of sepsis, particularly when interpreted alongside clinical signs and other laboratory findings. [21], [22]

A critical aspect of CRP kinetics is its delayed response; hence, it often does not reflect the early phases of neonatal sepsis comprehensively. Notably, CRP may remain within normal ranges in some infants even when significant systemic inflammation is present in the early stages of infection. Fortunately, subsequent elevations can offer prognostic insight, with higher levels often correlating with disease severity and worse clinical outcomes. In conjunction, evaluating the trend of CRP levels can further enhance diagnostic certainty and the effectiveness of intervention strategies, enabling clinicians to tailor antibiotic therapy more effectively. [21], [22]

Moreover, understanding the physiological role of CRP extends beyond mere diagnostic utility. CRP functions as a bridge between the innate immune response and inflammation. It facilitates opsonization and phagocytosis of pathogens, promoting their clearance from circulation. Additionally, CRP binds to damaged tissue and modulates the inflammatory response by activating complement pathways, thereby exerting a significant influence on inflammation resolution and tissue repair. This dual role illustrates CRP's importance, not only in diagnosing neonatal sepsis but also in

understanding the pathophysiology underlying systemic inflammatory responses in neonates. [4], [22], [23]

Recent studies emphasize the implications of systemic inflammation in neonatal outcomes, confirming that CRP levels are correlated with various complications, including sepsis-related morbidity and long-term neurodevelopmental disorders. For instance, elevated CRP levels have been associated with increased risk for neuroinflammation and potential cognitive deficits later in life in preterm infants. [3], [27] The exposure of the developing brain to systemic inflammation has been shown to disrupt critical neurodevelopmental processes, underscoring the need for careful monitoring and early intervention in neonates presenting with elevated CRP levels as a part of their clinical assessment.

Overall, the role of CRP as a biomarker for systemic inflammation and its consequential effects on neonatal health outcomes underscores the necessity for ongoing research into effective pathways for diagnosis, intervention, and management of neonatal sepsis. Understanding the inflammatory mechanisms in neonates is paramount not only for improving acute clinical care but also for mitigating long-term neurodevelopmental risks associated with systemic inflammatory conditions. [21], [22], [28]

1.3 Neurodevelopmental Consequences of Systemic Inflammation in the Neonatal Period

1.3.1 Brain Development During the Neonatal Window of Vulnerability

The neonatal period is characterized by remarkable brain development, marked by crucial processes such as neurogenesis, synaptogenesis, and myelination—each of which plays a foundational role in establishing a functional CNS. Neurogenesis, the generation of neurons from neural stem cells, occurs predominantly during this period, with the highest rates of neuronal proliferation seen in the early weeks of life. The newborn brain undergoes extensive synaptogenesis, a process during which

neurons form synapses to establish functional connections, laying the groundwork for future learning and cognitive capabilities. By the end of the second year of life, most neurons are formed, and synaptic connections begin to shape brain function. [29], [30] The process of myelination, where oligodendrocytes wrap axons in a myelin sheath to facilitate efficient signal transmission between neurons, follows closely behind. This crucial phase continues postnatally, contributing to improvements in cognitive and motor skills over the years.

During brain development, inflammation serves a dual role, having both physiological and pathological implications. While excessive inflammation is detrimental, moderate inflammatory responses are essential for proper brain maturation. During the neonatal phase, inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) facilitate critical processes including neuronal differentiation and survival and play roles in the maturation of synapses. [29], [30] These cytokines can promote the survival of certain neurons while encouraging the apoptosis of others, thus fine-tuning neuronal populations according to developmental needs. Importantly, short-lived inflammatory responses due to normal physiological insults or infections can trigger neurotrophic signalling, promoting resilience within neural circuits. However, when inflammation becomes dysregulated or chronic, it disrupts neurodevelopment, resulting in adverse outcomes such as cognitive impairments or increased risk for neurodevelopmental disorders. [5], [31]

The balance of pro-inflammatory and anti-inflammatory signals is indispensable during this window of plasticity. Evidence suggests that systemic inflammation, such as that instigated by infections or environmental toxins during this period, can have lasting effects on brain development, with documented instances of increased vulnerability to neurodevelopmental impairments post-exposure. Studies indicate that preterm infants—who are often subject to systemic inflammation due to invasive medical interventions—exhibit greater deficits in cognitive, motor, and behavioural outcomes as compared to full-term counterparts. [3], [31] Understanding these mechanistic pathways linking brain development to inflammatory processes can pave the way for targeted interventions to protect and

enhance neurodevelopment in at-risk populations, underscoring the importance of addressing systemic inflammation effectively in neonatal care.

1.3.2 Systemic Inflammation and Neuroinflammation: Pathophysiological Links

The interplay between systemic inflammation and neuroinflammation in neonates is critically influenced by cytokine signalling and microglial activity, two key mediators in this link. Upon activation, microglia, the resident immune cells of the CNS, modulate inflammatory responses and are pivotal in maintaining homeostasis within the brain.

Because of its close interaction with brain interstitial fluid, CSF can accurately reflect pathological changes in the CNS and is therefore an ideal source for assessing biochemical alterations related to neurological disorders [32], [33]. Cytokines released into circulation during systemic inflammation (for instance, during neonatal sepsis or conditions like necrotizing enterocolitis) can cross the BBB, leading to microglial activation and subsequent neuroinflammation. [30], [34] For this reason, some Authors have tested the use of CSF inflammatory markers as potential tools to distinguish bacterial meningitis from sepsis alone [35]. However, the interpretation of CSF parameters in neonates remains challenging, as its characteristics often overlap between sepsis and meningitis, limiting their diagnostic value [35].

IL-1β and TNF-α, two pro-inflammatory cytokines, have been indicated in studies as direct contributors to neuronal injury through various mechanisms, including apoptosis and oxidative stress, thereby leading to long-term neurodevelopmental consequences. [36], [37] Recent findings have illustrated that the oxidative stress resulting from heightened inflammatory cytokine levels can have damaging effects on neuronal health and myelination. For instance, reactive oxygen species (ROS) produced during inflammatory responses have been shown to perturb oligodendrocyte maturation and myelination—the disruption of which can lead to structural brain defects and cognitive impairments later in life. [29], [38] Furthermore, systemic inflammation can initiate a chain of events that not only

stimulates aberrant microglial activation but also alters their functional phenotype, shifting from protective to destructive roles. This maladaptive microglial behaviour can result in inappropriate synaptic pruning, leading to neurodevelopmental disorders. [30], [31]

Emerging evidence from clinical and experimental models underscores the strong association between neuroinflammation and negative neurodevelopmental outcomes. For example, infants who experience significant systemic inflammation during early life are at a heightened risk of developing cognitive deficits, with studies demonstrating correlations between elevated circulating cytokine levels and adverse developmental outcomes. [3], [29] There is a growing consensus that targeting the inflammatory process, whether through pharmacological interventions to mitigate cytokine signalling or through lifestyle modifications aimed at reducing environmental stressors, may hold promise for improving neurodevelopment in vulnerable neonatal populations. [39], [40] Additionally, investigating the mechanisms by which systemic inflammatory responses engage neurodevelopmental trajectories will be essential for developing comprehensive therapeutic strategies aimed at ameliorating the effects of neonatal inflammation and fostering optimal brain health. By elucidating the complex interplay between inflammatory mediators, microglial activation, and neurodevelopment, we can advance our understanding of pathological processes and ultimately enhance outcomes for neonates at risk due to inflammatory insults.

1.4 Brain Barrier Systems in the Neonate

1.4.1 Overview of Central Nervous System Barriers

The internal environment of the brain is defined and controlled by a series of mechanisms, usually referred to as the blood–brain barrier (BBB), which is highly efficient in maintaining the separation between the blood and central nervous system (CNS). More specifically, the BBB is a combination of highly specialized barrier systems which protect the CNS from fluctuations in blood composition, pathogens, and toxins.

Barrier systems are composed by different barrier interfaces, primarily the BBB and the blood-cerebrospinal fluid (CSF) barrier. [41] The BBB consists of brain endothelial cells lining CNS capillaries that are held together by tight junctions (TJ) [42], which lead to high electrical resistance and restrict paracellular diffusion to ensure that only selective substances can cross into the brain from the bloodstream. [43]. The junctional complex of TJs is formed by proteins, including occludin, claudins, and junctional adhesion molecules (JAM)- A, JAM-B, and JAM-C. [44]

This barrier effectively maintains a stable neuronal environment critical for maintaining proper synaptic transmission and overall brain function.

The blood-CSF barrier is primarily established by the TJ between the epithelial cells of the choroid plexus. [43], [45] Choroid plexus cells are epithelial cells which have TJ at the apices; these form the structural basis of the blood-CSF barrier, preventing the penetration of even small molecules from the blood to the CSF. [46] The blood vessels in the stroma of the choroid plexus are fenestrated and do not form a proper barrier, although their endothelial cells appear to contain key efflux transporters that prevent the entry of many lipid-soluble molecules into the brain and CSF. The blood-CSF barrier regulates the composition of CSF, which plays essential roles in nutrient delivery, waste removal, and cushioning the brain, thus maintaining homeostasis within the CNS.

Other barrier mechanisms include circumventricular organs [47], strap junctions between adjacent neuroepithelial cells (embryonic CSF-brain barrier) [48], which restrict all but the smallest lipid-insoluble molecules from entering the brain [49], [50], and the arachnoid barrier, whose TJ restrict the permeability between the fenestrated blood vessels in the dura and subarachnoid space. The blood vessels within this space have TJ between the endothelial cells but no astrocytic end feet or pericytes. [33]

Functionally, the BBB exhibits a high degree of selectivity, preventing the passage of large or hydrophilic molecules while allowing essential nutrients, ions, and small lipophilic substances to diffuse freely. In contrast, the blood-CSF barrier is involved in the active transport of substances into the CSF, functioning somewhat differently as it participates in both the secretion of certain proteins

and the removal of waste products from the CSF. [51] The functional attributes of these two barriers underline their distinct but complementary roles in the protection and nourishment of the developing brain. This understanding is crucial, as the immaturity of these systems renders neonates more susceptible to neurotoxic substances, pathogens, and systemic inflammatory processes, highlighting the need for protective interventions. [51], [52]

1.4.2 Structural and Functional Features of the Blood–Cerebrospinal Fluid Barrier

The blood-CSF barrier is primarily formed by the highly vascularized choroid plexus, which is composed of specialized epithelial cells that interconnect tightly via tight junctions. [53] These junctions are critical for facilitating the barrier's regulatory functions; they limit the passive diffusion of substances and act as sites for specific transporter proteins that can actively transport molecules across the barrier. For instance, ATB-binding cassette (ABC) transporters, such as P-glycoprotein and multidrug resistance protein 1, are prominent at the blood-CSF barrier and facilitate the efflux of potentially harmful substances from the CSF back into the blood. [53], [54] Recent research has highlighted that the choroid plexus is not merely a passive barrier but an active environmental regulator of the CSF that participates in various functions, including immune surveillance and metabolic activities. [55]

The structural integrity of tight junctions, composed of proteins like occludin and claudins, is pivotal for maintaining the blood-CSF barrier, ensuring that CSF has a specific composition crucial for brain development and function. [53], [56] Abnormalities in tight junction dynamics can lead to disruptions in CSF composition and brain homeostasis, significantly impacting neonatal health. Furthermore, the choroid plexus expresses diverse transport proteins fundamental for ion and nutrient transport, such as the sodium-potassium ATPase, which is vital for maintaining ionic gradients essential for normal neuronal activity. [57] Understanding these structural features and their implications in regulating

CNS homeostasis during the early stages of life can provide critical insights into developing strategies for treating conditions that challenge BBB and blood-CSF barrier integrity.

1.4.3 Maturation and Plasticity of the Blood–Cerebrospinal Fluid Barrier in Early Life

The maturation of the blood-CSF barrier is a dynamic process that unfolds throughout the early postnatal period. Initially, this barrier exhibits considerable plasticity, wherein the functions of transport and permeability are finely tuned in response to the rapidly changing needs of the developing brain. [58]

It has been widely believed for nearly a century that barrier mechanisms in the fetal and newborn brain are absent or poorly developed. [59], [60], [61] This belief appears to stem from misunderstandings and mistranslations of early studies (some of which were characterized by poor experimental design [62]) which used terms that imply some level of dysfunction like “inefficient” [63] and “primitive” [64], thus suggesting a functional deficiency. However, differences in barrier mechanisms between developing and adult brains may reflect an appropriate adaptation to the specific developmental stage. [41] We support the theory that these mechanisms are not underdeveloped but finely tuned to the specific developmental needs of the CNS at each stage of fetal growth. This maturation trajectory is crucial as it supports the accumulation of neurotrophic factors and essential compounds while preventing neurotoxic substances from reaching the CNS.

Neonates, particularly those born preterm, often demonstrate altered blood-CSF barrier function, which can be exacerbated by stressors such as systemic inflammation or infection. [65], [66] Enhanced permeability during this period can lead to increased vulnerability to neurodevelopmental disorders and acute brain injuries. Understanding the adaptability of the blood-CSF barrier allows for identifying critical windows of intervention where therapeutic approaches may mitigate adverse outcomes associated with immune system challenges. For instance, augmenting nutrient transport across the blood-CSF barrier while simultaneously protecting against neurotoxic exposure presents a

fascinating area for future research and could lead to advancements in clinical strategies aimed at improving outcomes for at-risk newborns. Moreover, the relevance of the blood-CSF barrier in the context of drug delivery mechanisms continues to be a significant area of exploration. As novel therapeutic agents are developed, identifying pathways through the choroid plexus may enhance pharmacokinetics and efficacy in treating diseases affecting the CNS, such as neonatal sepsis or neuroinflammatory conditions. [67], [68]

The unique and adaptable characteristics of the blood-CSF barrier in neonates emphasize its vital role in protecting brain integrity while also serving as a potential target for therapeutic interventions aimed at alleviating the long-lasting consequences of systemic inflammation and infection in neonates. Understanding brain barrier systems in neonates—including their structural, functional, and developmental features— could provide to critical insights into the complexities of neonatal brain health. By elucidating the mechanisms underlying the BBB and blood-CSF barrier, researchers and clinicians could better address the challenges associated with maintaining CNS homeostasis in vulnerable populations, paving the way for innovative strategies that promote neuroprotection and improve overall developmental outcomes.

1.5 Systemic Inflammation and Blood–Cerebrospinal Fluid Barrier Modulation

1.5.1 Inflammatory Mediators and Peripheral–Central Communication

Systemic inflammation is characterized by the release of various inflammatory mediators, including cytokines such as IL-1, IL-6, and TNF-α. These cytokines act as chemical messengers that facilitate communication between the periphery and the CNS, effectively linking immune responses to neural function. [69] Following an inflammatory stimulus, these cytokines are produced by peripheral immune cells and can influence numerous physiological processes within the CNS. Some cytokines can cross the BBB and the blood-CSF barrier, thus directly inducing neuroinflammation and altering the homeostasis of the neural environment. For instance, IL-1 and TNF-α have been shown to induce

a response in glial cells, leading to increased production of reactive oxygen species and nitric oxide, which can further exacerbate inflammation and neuronal injury. [70], [71]

The transport mechanisms through which cytokines modulate this communication can be classified into active and passive transport. Active transport mechanisms involve specific transport proteins that facilitate the translocation of larger or charged molecules across barrier systems, while passive transport allows for straightforward diffusion across the barriers for smaller lipophilic molecules. [72] Cytokines often employ these transport systems to exert their effects within the CNS. The expression levels of transporters such as the glucose transporter and various ATP-binding cassette transporters can change during states of inflammation, thereby altering the availability of key metabolic substrates essential for neuronal function and survival. [73] Moreover, the dysregulation of these transport mechanisms in the context of systemic inflammation can lead to compromised barrier integrity, resulting in increased permeability that may allow for the passage of potentially neurotoxic elements into the CNS. [74]

Understanding the dynamics of inflammatory mediators and their transport mechanisms elucidates the complex interplay between peripheral and central inflammation. Enhanced CNS inflammation due to elevated cytokine levels during systemic inflammatory conditions can lead to significant neurodevelopmental consequences, particularly in vulnerable populations such as neonates. [3], [75] Thus, examining these pathways is vital in formulating therapeutic interventions aimed at mitigating inflammation-related neural dysfunction without disrupting the delicate balance of central homeostasis.

1.5.2 Cerebrospinal Fluid as a Mirror of Central and Peripheral Inflammatory Processes

CSF acts as an essential medium for communication within the CNS, reflecting both local and systemic inflammatory processes. This dynamic compartment plays a pivotal role in maintaining a stable environment for neuronal function while also serving as an influential reservoir of signalling

molecules that can influence neurodevelopment and health. The composition of CSF is not static but responds dynamically to various physiological and pathological conditions. [76] For instance, during systemic inflammation, alterations in CSF protein concentrations, including the presence of inflammatory cytokines and chemokines, can indicate ongoing peripheral inflammatory processes that have implications for CNS activity.

Changes in the biochemical composition of the CSF—such as altered levels of neural proteins, cytokines, and immune mediators—can serve as biomarkers for assessing the inflammatory state of the CNS. Inflammatory events, particularly those associated with conditions like sepsis or neuroinflammatory disorders, elicit changes in the CSF signature, characterized by increased levels of proteins like glial fibrillary acidic protein (GFAP) or S100 calcium-binding protein B (S100B), both of which can signify astrocytic activation and brain injury. [74], [77] Such changes in CSF dynamics underscore its utility not only as a diagnostic tool but also as a window into the state of neuroinflammation, providing insights into the underlying mechanisms linking systemic and central inflammatory pathways.

Furthermore, the imaging of CSF dynamics using techniques such as phase-contrast magnetic resonance imaging (MRI) has revealed the influence of physiological and pathological processes on CSF flow and volume. [78] Normal physiological conditions maintain a stable composition, but during episodes of systemic inflammation, the barrier functions regulating CSF homeostasis may become compromised, leading to enhanced entry of leukocytes and inflammatory mediators which propagate local inflammatory responses within the brain. [3] It is through understanding these alterations that one can discern the delicate balance of neuroinflammation and its consequences, particularly in neonates who may exhibit altered responses to systemic stressors.

1.5.3 Evidence of Barrier Dysfunction in Neonatal Inflammatory Conditions

The integrity of the blood-CSF barrier is crucial in protecting the CNS from the repercussions of systemic inflammation. Emerging evidence highlights barrier dysfunction in the context of neonatal inflammatory conditions, such as sepsis or significant infections, which can exacerbate the risk of neurodevelopmental impairments. [75] In this context, CNS injury would result from not only the bacterial cytotoxicity, but also through adverse systemic inflammation (even in the absence of a direct invasion of CNS by the pathogen organism) or altered brain perfusion in the setting of hemodynamic instability. [79], [80]

Increasing evidence indicates that barrier systems integrity is compromised during sepsis. Preclinical animal and *in vitro* studies have demonstrated that exposure to pro-inflammatory cytokines can lead to substantial morphological and functional alterations in blood-CSF barrier integrity, resulting in increased permeability. For instance, barrier permeability resulted increased in the cerebral cortex, perirhinal cortex, hippocampus, and thalamus of rats after a single intraperitoneal injection of lipopolysaccharide, the major component of the outer membrane of Gram-negative bacteria, when compared to saline-treated controls; [81] disruptions in TJ between choroid plexus epithelial cells have been associated with elevated CSF protein concentrations and immune cell infiltration. [3] In humans, brain tissue samples obtained from deceased sepsis patients revealed a significant down-regulation of the TJ proteins occludin, claudin-5, and zonula occludens-1 in microvascular endothelial cells [82], suggesting impaired barrier function, which would contribute to the pathophysiology of sepsis, as the CNS becomes highly vulnerable to neurotoxic factors such as free radicals, inflammatory mediators, intravascular proteins, plasma, and circulating leukocytes [83], [84], [85]. Consequently, barrier deficiencies lead to brain edema formation and reduced microvascular perfusion, contributing to and exacerbating neuronal loss [83] and subsequent neurodevelopmental impairment in infants. [86], [87], [88] [89]

Despite these advances, the literature regarding the mechanisms underpinning blood-CSF barrier dysfunction in neonates remains limited. Many studies focus primarily on adult animal models, highlighting a critical gap in our understanding of how systemic inflammation uniquely affects the neonatal blood-CSF barrier. [90] Variability in experimental conditions and the reliance on animal models may not fully replicate the complexities observed in human neonates, complicating the translation of findings to clinical settings. Therefore, there remains a pressing need for research that characterizes the nature of blood-CSF barrier dysfunction during distinct phases of neonatal inflammatory conditions, particularly in the context of clinical interventions designed to optimize outcomes in vulnerable infants. By identifying specific cytokine profiles and their impact on barrier functionality, clinicians may better tailor treatments to reduce the long-term neurodevelopmental consequences of early-life inflammation, enhancing the odds of improved outcomes for affected neonates.

1.6 Proteomic Approaches to Study Neonatal Inflammation and Brain Barrier Function

1.6.1 Principles of Proteomics in Biomedical Research

Proteomics is a comprehensive field of study focused on the large-scale analysis of proteins, particularly in understanding their functions, structures, and interactions within biological systems. At its core, proteomics aims to identify and quantify proteins in complex biological samples, shedding light on the molecular underpinnings of various physiological and pathological conditions. This discipline is particularly valuable in biomedical research, where understanding protein expression patterns can reveal important insights into disease mechanisms, therapeutic targets, and potential biomarkers for diagnostic purposes.

Key techniques in proteomics include mass spectrometry (MS), two-dimensional gel electrophoresis (2DGE), and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). With the

advent of high-resolution MS and bioinformatics tools, researchers can now analyse thousands of proteins simultaneously, enabling the generation of detailed protein maps that represent cellular states under varying conditions. By employing these methods, researchers can delineate protein profiles that reflect different biological scenarios, such as inflammation, neurodegeneration, or responses to treatment. Importantly, the proteomic approach is not limited to qualitative assessments but enables quantitative comparisons across different states, providing insights into regulatory mechanisms governing protein synthesis and breakdown.

Despite its numerous advantages, proteomics faces inherent challenges, such as sample complexity and the dynamic nature of the proteome, influenced by numerous factors including developmental stage, environmental changes, and disease states. Careful experimental design, proper sample handling, and robust bioinformatic analyses are essential for generating reproducible and meaningful results. Through these efforts, proteomics provides a powerful toolkit for deciphering the biological roles of proteins in neonates, particularly in understanding how systemic inflammation influences brain barrier function and overall neurological health.

1.6.2 Proteomic Profiling of Blood and Cerebrospinal Fluid in Neonates

In neonates, proteomic profiling of blood and CSF has emerged as a valuable approach for characterizing the inflammatory environment and assessing brain barrier integrity during critical periods of development. Because of its close interaction with brain interstitial fluid, CSF can accurately reflect pathological changes in the CNS and is therefore an ideal source for assessing biochemical alterations related to neurological disorders [32], [33]. Indeed, proteomic profiling of CSF has already identified biomarkers in several neurological conditions, including Alzheimer's disease [91], dementia [92], multiple sclerosis [93], and septic encephalopathy [94]. Yet, systematic proteomic analyses of CSF in neonates with LOS are still lacking. Previous studies have highlighted the presence of distinct proteomic signatures in the CSF of neonates suffering from various

conditions, including sepsis and hypoxia-ischemia, which can serve as important biomarkers of inflammation and neuronal injury. [5], [69], [95] For instance, studies have identified changes in levels of neuroinflammation-related proteins such as neuropeptide Y, matrix metalloproteinases, and cytokines in the CSF and plasma of preterm infants following bacterial infection, suggesting that these proteins can inform us about the neurological status and potential outcomes in this vulnerable population. [5], [96], [97]

The identification of both well-established and emerging biomarkers through proteomic profiling offers a deeper understanding of the processes involved in neonatal inflammation. For example, an increase in S100B, a neurotrophic protein, in CSF has been associated with complications in preterm infants, indicating its potential as an early marker for brain injury. Conversely, pro-inflammatory cytokines like IL-6 in CSF have shown mixed associations with neurodevelopmental outcomes, reflecting the complex nature of the neonatal inflammatory response. [98], [99] Additionally, the dynamic changes in the protein composition of CSF during early life assert its role as a robust indicator of both central and peripheral inflammatory processes that can influence neurodevelopment. Future studies should focus on standardizing proteomic methodologies to facilitate comparability among cohorts and validation of novel biomarkers across different clinical scenarios, as this would not only enhance diagnostic accuracy but also provide insights into potential therapeutic interventions. The evolving landscape of proteomic technologies, such as MS and liquid chromatography, continues to push the boundaries of our understanding of neonatal health, ultimately paving the way for improved strategies in managing conditions associated with inflammatory responses.

1.6.3 Proteomics as a Tool to Investigate Blood-CSF Barrier Dynamics

The blood-CSF barrier plays a crucial role in maintaining the brain's microenvironment, and proteomics serves as a powerful tool to investigate the dynamics of this barrier under various

physiological and pathological conditions. Proteins such as claudins, occludin, and various transporters are integral components of the blood-CSF barrier, defining its permeability and transport characteristics. Notably, alterations in the expression patterns of these barrier proteins can indicate changes in barrier integrity, often in response to systemic inflammatory processes or injury. [100], [101] For instance, an upregulation of claudin-5 in CSF correlates with BBB dysfunction following neonatal hypoxic-ischemic injury, which offers the possibility of using such proteins as biomarkers to assess brain barrier integrity in clinical settings. [101], [102]

Understanding the interplay between protein transport mechanisms and permeability at the blood-CSF barrier is essential for elucidating the impact of both endogenous and exogenous factors on the neonatal brain. Proteomic approaches can help identify transport proteins that facilitate the movement of essential nutrients and signalling molecules across this barrier, ultimately influencing neuronal health and function. Moreover, dynamic changes in the permeability of the blood-CSF barrier during systemic inflammation can lead to increased exposure of the CNS to potentially harmful substances, exacerbating inflammatory responses and neuronal injury.

Further research is necessary to explore how disruptions in blood-CSF barrier dynamics manifest at the protein level during conditions of neonatal sepsis and other inflammatory states. Integrating proteomic profiling with functional studies on barrier permeability will shed light on potential therapeutic targets, either by enhancing protective mechanisms or by developing strategies to mitigate injury during critical periods of neuronal development. A comprehensive understanding of blood-CSF barrier dynamics through proteomics will ultimately contribute to improved neonatal care approaches and therapeutic interventions aimed at fostering healthy brain development in vulnerable infants.

In conclusion, proteomic approaches to studying neonatal inflammation and brain barrier function offer a promising avenue for understanding the complexities of neurodevelopment in the context of systemic inflammation. By elucidating the roles and behaviours of key proteins in blood and CSF, researchers can enhance their understanding of the biological underpinnings of brain health, paving

the way for improved diagnostic and therapeutic strategies aimed at optimizing outcomes for neonates exposed to inflammatory challenges.

1.7 Rationale and Objectives of the Present Study

1.7.1 Knowledge Gaps in the Study of Neonatal Inflammatory Syndromes

Research on neonatal inflammatory syndromes highlights critical knowledge gaps regarding the complex interactions between systemic inflammation and CNS integrity. While significant strides have been made in understanding adult inflammatory processes, the neonatal brain exhibits unique characteristics and vulnerabilities due to its immature developmental status and evolving neural architecture. The BBB and blood-CSF barrier play essential roles in regulating the composition of the CNS microenvironment; however, their specific responses to systemic inflammatory conditions in neonates remain poorly elucidated. This underscores a crucial gap in our understanding of how neonatal brain barriers might facilitate or inhibit the entry of inflammatory mediators, potentially leading to neurodevelopmental outcomes later in life.

Moreover, while cytokines and other inflammatory mediators are known to influence neuronal function and viability, limited research has systematically characterized their effects within the context of neonatal CSF and blood, complicating the identification of reliable biomarkers for early detection of neuroinflammatory conditions. Existing literature often relies on mixed-age populations, neglecting the unique ontogeny of the neonatal inflammatory response, which may differ considerably from that of older children or adults. This oversight presents a significant barrier to effectively tailoring therapeutic strategies to mitigate the adverse effects of inflammation in neonates. The engagement of multi-faceted proteomic approaches capable of delineating nuanced molecular signatures related to inflammation in neonates is thus critical for bridging these gaps in knowledge and enhancing our understanding of neonatal health and disease.

1.7.2 Study Rationale: A Proteomic Comparison of Blood and Cerebrospinal Fluid in Neonates with Systemic Inflammation

The present study's rationale originates from the necessity to utilize a proteomic lens to investigate the effects of systemic inflammation on both blood and CSF in neonates. Proteomics offers a powerful methodological framework for assessing broad changes in protein expression and functionality in response to systemic inflammatory events. By comparing protein profiles between blood and CSF, the study aims to elucidate the dynamic interactions occurring at the blood-brain interfaces and how these contribute to or mitigate the effects of inflammation on neurodevelopment.

Prior research has established that the composition of CSF, being sensitive to perturbations in systemic homeostasis, can reflect pathological conditions impacting the brain. However, it remains unclear how these changes correlate with specific systemic inflammatory responses, particularly in neonates. Investigating these relations through proteomics will enable the identification of potential biomarkers that could guide clinical decision-making regarding neuroprotective strategies in at-risk neonate populations. Additionally, understanding the differential protein expressions in blood versus CSF could unveil insights into the pathways governing immune responses within the CNS and potentially highlight new therapeutic targets.

The integration of proteomic analyses in this study will focus on quantifying critical proteins involved in neuroinflammatory pathways, transport mechanisms at the blood-CSF barrier, and cytokine profiles that inform about the systemic and central nervous influences during inflammation. This comprehensive approach promises to provide invaluable insights into how systemic inflammatory states translate into neurobiological outcomes for neonates, an endeavour that could significantly impact pediatric care and intervention strategies.

1.7.3 Hypothesis and Study Objectives

Taken together, the evidence discussed so far highlights a critical gap in our understanding of how systemic inflammation during LOS interacts with the CNS. While clinical and experimental data suggest an association between LOS and adverse neurodevelopmental outcomes, the biological mechanisms underlying this relationship -especially in the absence of overt CNS infection- remain largely unresolved. Moreover, increasing attention has been directed toward the role of brain barrier systems, and particularly the blood-CSF barrier, as a dynamic interface capable of modulating peripheral-central inflammatory crosstalk.

Based on these premises, the present thesis was designed with three main objectives:

1) To provide *in vivo* evidence of proteomic modulation of temporally matched plasma and CSF samples of term and preterm infants across different stages of systemic inflammation, and to relate these changes to CRP levels, widely used as a quantitative biomarker of infection and inflammation.
2) To explore potential biological mechanisms of neurological involvement occurring after LOS, in the absence of biochemical and microbiological evidence of CNS infection.
3) To investigate, in vivo, the functional state and integrity of the blood-CSF barrier in newborns with sepsis.

By addressing these objectives through a combined CSF-plasma proteomic approach, this work aims to contribute to a more integrated understanding of neonatal neuroinflammation and brain barrier physiology during systemic infectious stress. Furthermore, providing *in vivo* evidence of preserved blood-CSF barrier integrity during LOS may help to counter the longstanding misconception of an intrinsically deficient barrier function in newborns, a concept widely propagated in the literature for more than a century. [62], [103] Ultimately, the study also seeks to refine the biological interpretation of plasma CRP levels in the context of neonatal LOS.

2. Methods

2.1 Sample collection and clinical data

The sample collection was conducted at the Neonatal Intensive Care Unit of Gaslini Children's Hospital, Genoa, Italy between January 2023 and March 2024. All patients with clinical LOS (>72 h of life) who were screened for meningitis with a lumbar puncture (LP) for CSF examination – according to internal protocol - were eligible. Based on experienced neonatologists' evaluation, suspected LOS was defined based on a deterioration of clinical conditions, characterized by ≥1 of the following: occurrence of subsequent non-obstructive apneas, hyporeactivity, grey/marbled skin colour, or a significant increase in heart-rate observation monitoring. Venous blood sampling and LP were performed exclusively for clinical purposes, according to institutional protocols for the evaluation of suspected LOS. For research purposes, an additional aliquot of approximately 1 mL of blood and 0.5 mL of CSF were collected at the time of sampling, without requiring additional procedures. Blood was collected in an ethylenediaminetetraacetic acid (EDTA) tube, and LP was performed within 30 minutes. Serum CRP values were obtained through immunoassay analysis in the Central Analysis Laboratory department of Gaslini Children's Hospital.

CSF samples with a volume <0.2 mL, contaminated with blood, or centrifuged beyond 12 h after collection were discarded. Plasma was isolated by centrifugation for 15 min at 1200 g/2800 RPM using a laboratory centrifuge (MPW M-BASIC, MPW Med. Instruments, Warsaw, Poland). Both plasma and CSF samples were stored in a HERA Freeze HFC refrigerator (Heraeus Holding GmbH, Hanau, Germany) at -80 °C within 3 h of collection and later transferred to laboratories in refrigerated bags. Plasma samples with evidence of haemolysis after centrifugation were discarded.

Anonymized clinical information of the included infants, such as demographics and results from haematological and biochemical tests of blood and CSF, was also retrieved from the hospital's electronic medical records.

Systemic inflammation was defined by a positive CRP at first detection or control (performed within 12 and 18 h). Despite a clinical picture compatible with sepsis, patients with a persistently negative CRP level (CRP group 1 – "not inflamed" group) were classified as the non-inflamed population. To shed light on the clinical and biochemical differences related to an increasing "amount of inflammation", the CRP-positive population was further divided into two incremental stages of systemic inflammation as follows: CRP group 2 = negative CRP at onset, positive CRP at control – "evolving inflammation" group; CRP group 3 = positive CRP at both timepoints – "overt inflammation" group.

Proteomic analysis

Sample preparation. Proteomic analyses of CSF and plasma samples were performed by high-resolution mass spectrometry (MS). For CSF, protein concentration was measured with the Qubit Protein Assay Kit (Thermo Scientific) and 30 μg of proteins were used. For plasma, 3 μL of sample were diluted in 60 μL of lysis buffer and the equivalent of 30 μg of proteins was processed. In both matrices, proteins were denatured, reduced, and alkylated with iST-LYSE buffer (PreOmics) for 10 min at 95 °C on a ThermoMixer (1000 rpm). Protein Aggregation Capture (PAC)-based protein isolation and digestion [104] were automated on a KingFisher™ Apex system (Thermo Scientific). Proteins were precipitated onto magnetic beads by adding acetonitrile to a final concentration of 70% and sequentially washed with acetonitrile, ethanol, and isopropanol to remove contaminants. On-bead digestion was carried out in 25 mM Tris buffer (pH 8.0) containing trypsin (0.7 μg, Promega) and Lys-C (0.3 μg, Wako) at 37 °C for 2.5 h. Protease activity was quenched by acidification with 2% trifluoroacetic acid (TFA), and peptides were loaded onto Evotip Pure (Evosep Biosystems) for LC-MS/MS analysis.

LC/MS-MS analysis. Peptide mixtures were analysed on an Evosep One system coupled to an Orbitrap Exploris 480 mass spectrometer equipped with a FAIMS Pro Duo interface (Thermo

Scientific). Peptides were separated on an EASY-spray C18 column (150 μm × 15 cm, Thermo Scientific) using the pre-programmed 30 samples/day gradient, with a flow rate of 500 nL/min and column temperature at 40 °C. MS analysis was performed in data-independent acquisition (DIA) mode with positive polarity. Full MS scans were acquired at 120,000 resolution (375–1500 m/z, AGC target 300%, injection time Auto), and MS/MS scans were recorded with 40 windows of 15 Da (1 Da overlap) at 30,000 resolution (AGC target 1000%, injection time Auto, normalized collision energy 30%).

Data analysis. Raw data were processed with Spectronaut v18 (Biognosys AG) in directDIA mode using default settings against the UniProt Human database (downloaded November 2022). A 1% false discovery rate (FDR) was applied at peptide and protein levels, and quantification values were normalized using the Spectronaut cross-run algorithm. Processed data were exported as a Protein Quant Pivot Report and imported into Perseus v1.6.5.0 [105] for downstream analyses.

For statistical analysis, proteins were retained if at least 70% of valid values were present in one experimental group, and missing values were imputed from a normal distribution (downshift 1.8, width 0.3). Differential protein expression among CRP-based groups was assessed by one-way ANOVA, followed by Tukey's post hoc test for pairwise comparisons with permutation-based FDR correction (FDR < 0.05). For haemoculture-based comparisons, Student's t-test was applied ($s_0 = 0.1$, permutation-based FDR < 0.05). Functional enrichment was evaluated by gene set enrichment analysis (GSEA) on the full ranked dataset and by over-representation analysis (ORA) restricted to significant DEPs, using Gene Ontology (GO) Biological Process categories. Protein–protein interaction (PPI) networks were generated with the stringApp database and visualized in Cytoscape (v3.10.3), with node size proportional to statistical significance and edge thickness reflecting interaction confidence. Statistical analyses were conducted in Perseus (v1.6.15), while data visualization was performed in R (v4.4.3) with the ggplot2, ComplexHeatmap and VennDiagram packages. Gene set enrichment analysis (GSEA) was performed using the clusterProfiler, msigdbr, and enrichplot R packages.

Statistical analysis

Descriptive statistics were performed, with continuous variables expressed as mean ± standard deviation (SD) or median and range, and categorical variables as absolute and relative frequencies. Group comparisons were made using one-way ANOVA with Tukey's post hoc test for protein expression, the Mann-Whitney U-test for continuous variables, and χ^2 test or Fisher's exact test for categorical variables. All p-values were calculated using two-sided tests, with a statistically significant p-value < 0.05. Statistical analysis was performed using SPSS for Windows version 29 (IBM SPSS Inc., New York, NY, USA).

Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [106] partner repository with the dataset identifier PXD068770.

3. Results

3.1 Statistical analysis

Nine patients were discarded due to contamination by haemolysis or delayed centrifugation (>12 h) of CSF and plasma samples. CSF and plasma samples from a total of 45 neonates were analysable. Patients showed a mean [SD] GA at birth of 30.52 weeks; 31.1% (N = 14) were females and 68.9% (N=31) were males, with an average birth weight (BW) of 1495 g ± 814 g; no significant difference in GA at birth was found between CRP-based groups.

Sepsis investigations were conducted at a median of 33.8 weeks of cGA and at a median of 21 days of life, with a significant difference between cGA between CRP group 1 and the other two CRP-based groups (CRP group 1 average cGA at symptoms of LOS = 31.36 weeks *versus* CRP group 2 cGA at

symptoms of LOS = 36.34 weeks, p = 0.005, and CRP group 1 average cGA at symptoms of LOS = 31.36 weeks *versus* CRP group 3 cGA at symptoms of LOS = 35.40 weeks, p = 0.009). A significantly lower 1st minute Apgar and GA at antenatal steroid administration were found. A significant difference between the average birth weight (BW) centile in CRP group 1 and 3 was found (58.17 *versus* 30.20 respectively, p = 0.01).

At the time of plasma and CSF samples collection, 16 patients (36.3%) had an epicoutaneous-caval catheter, 4 (9.1%) had an umbilical venous catheter, and 4 (9.1%) had a central venous catheter, with an average time from placement of 8 days.

Only 14 patients (31.1%) had a haemoculture positivity. Most of them (71.4%) were interpreted as an haemoculture contamination by Gram-positive skin organisms (6 *Staphylococcus epidermidis*, 3 *Staphylococcus haemolyticus*) or orofecal transmitted Gram-negative organisms with a late growth in haemoculture (1 *Salmonella* spp.). Due to the haemoculture positivity for *Klebsiella* spp (1 *Klebsiella pneumoniae*, 1 *Klebsiella oxytoca*), *Escherichia coli* and methicillin-resistant *Staphylococcus aureus*, 4 patients (28.6% of haemoculture-positive infants) continued antibiotic therapy for 14 days. None of the patients had a subsequent positivity in a control haemoculture. 21 of the patients (46.7%) showed repeatedly negative CRPs. No significant difference in the average higher CRP value was found between CRP group 2 and 3.

Average white blood cell (WBC) count resulted higher in CRP group 1 *versus* CRP group 2 (11.97 el/μL *versus* 8.43 el/μL, p = 0.005), thus reflecting the importance of the WBC reduction as an early manifestation of neonatal LOS. Interestingly, no significant difference was found between CRP group 1 and 3; we state that the latter could represent a more developed stage of systemic inflammation, which manifests itself, among other aspects, with a pseudo-normalisation of the WBC.

No significant difference was found in glycemia and CSF glucose level between the three groups. No significant difference was found in BW at 36 weeks of cGA, weeks of cGA and BW at discharge, and NICU stay between the three groups.

Results in the three plasmatic CRP-based groups are summarized in Tables 1 and 2.

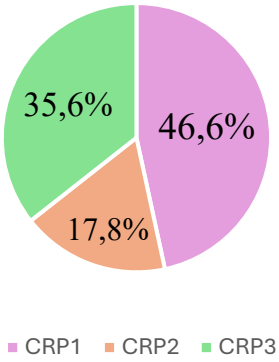
	CRP1	**CRP2**	**CRP3**	**CRP1 vs CRP2**	**CRP1 vs CRP3**	**CRP2 vs CRP3**
	N=21	N=8	N=16			
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	*p* value	*p* value	*p* value
GA at birth, weeks	29.11±2.95	31,45±5,03	31,92±4,80	0.24	0.08	0.83
BW at birth, g	1290,5±650	1621,3±972,9	1700,9±910,7	0.55	0.19	0.74
BW centile	58.17±32.65	37.5±25.84	30.20±21.78	0.12	**0.01**	0.56
GA at the administration of antenatal CS, weeks	25,94±2,36	28,94±3,19	29,51±3,32	0.12	**0.03**	0.69
Apgar 1° minute	4,95±2,56	6,38±2,13	6,73±1,67	0.18	**0.03**	0.73
Apgar 5° minute	7,62±1,43	8,38±0,92	8,47±1,06	0.28	0.10	0.78
GA at spontaneous breathing, weeks	36,02±2,77	37,45±3,18	36,22±1,99	0.24	0.85	0.40
CRP at symptoms, ng/dl	0±0	0±0	2,54±2,15	1	**<0.001**	**<0.001**
Highest CRP value, ng/dl	0±0	3,22±4,53	3,38±3,13	**<0.001**	**<0.001**	0.19
Glycaemia, mg/dl	103,62±22,73	88,43±12,19	91,07±30,56	0.07	0.08	0.41
Neutrophils count, el/μL	3,93±2,67	3,70±1,57	7,49±6,05	0.90	0.10	0.14
WBC, el/μL	11,97±2,88	8,43±2,58	12,70±7,64	**0.005**	0.68	0.18
cGA at symptoms of LOS, weeks	31,36±2,54	36,34±4,21	35,40±5,06	**0.005**	**0.009**	0.55
Days of life at symptoms of LOS	15,10±7,82	34,25±27,48	23,13±16,13	0.14	0.11	0.53
Days from catheter placement	6.18±3.28	12.50±3.54	9.20±5.53	0.10	0.25	0.48
CSF cell count, el/μL	65,70±196,20	4,25±4,23	49,06±173,69	0.41	0.67	0.65
CSF glucose, mg/dl	54,55±17,26	55,25±11,04	56,19±18,72	0.67	0.81	0.88
BW at 36 weeks cGA, g	2066,1±355,6	1911,2±135,4	1930±354,8	0.17	0.27	1
cGA at discharge, weeks	39,95±3,34	41,45±2,96	43±4,39	0.28	0.97	0.27
BW at discharge, g	2904,2±549,3	2813,3±495,3	2806,6±620,3	0.70	0.68	0.97
NICU stay	77,30±26,17	50,86±34,60	55,60±31,23	0.09	0.02	0.78

Table 1 Continuous variables of the three plasmatic CRP-based populations. CRP groups were defined as follows: CRP1 = negative CRP at onset and control (12–18 h); CRP2 = negative at onset, positive at control; CRP3 = positive at both timepoints.

	CRP1	**CRP2**	**CRP3**	**CRP1 vs CRP2**	**CRP1 vs CRP3**	**CRP2 vs CRP3**
	N=21	N=8	N=16			
	N (%)	**N (%)**	**N (%)**			
Gender, M	17 (19)	4 (50)	10 (37.5)	0.16	0.27	0.67
IUGR, yes	3 (14,3)	1 (14,3)	5 (33,3)	1	0.24	0.62
Complete pre-natal CS administration, yes	18 (85,7)	5 (62,5)	9 (56,3)	0.30	0.07	1
Presence of a vascular access, yes	20 (95,2)	5 (62,5)	14 (87,5)	**0.05**	0.57	0.29
BPD, yes	11 (55,0)	5 (62,5)	7 (46,7)	1	0.74	0.67
PDA, yes	11 (52,4)	4 (50,0)	4 (26,7)	1	0.18	0.37
NEC, yes	0 (0)	0 (0)	0 (0)	-	-	-
ROP, yes	4 (20,0)	1 (12,5)	4 (25,0)	1	1	0.63
Haemoculture positivity for a non-contaminant germ, yes	0 (0)	1 (12,5)	2 (12,5)	0.28	0.18	1
CSF culture positivity, yes	0 (0)	0 (0)	0 (0)	-	-	-
CSF >20 el/μL	5 (23,8)	0 (0)	2 (12,5%)	0.28	0.67	0.54
CSF glucose <40 mg/dl	5 (23,8)	1 (12,5)	4 (25,0)	0.65	1	0.63
CSF protein 60-150 mg/dl	14 (66,7)	6 (75,0)	7 (43,8)	1	0.20	0.21
CSF protein >150 mg/dl	7 (33,3)	1 (12,5)	6 (37,5)	0.38	1	0.35

Table 2 Categorical variables of the three plasmatic CRP-based populations. CRP groups were defined as follows: CRP1 = negative CRP at onset and control (12–18 h); CRP2 = negative at onset, positive at control; CRP3 = positive at both timepoints.

735 Division of the patients in the three plasmatic CRP-based groups is described in the graph below.



736

737

Group type	Subgroup	CSF (n)	Plasma (n)
CRP-based	CRP1	15	6
	CRP2	6	5
	CRP3	14	11
	Total	**35**	**22**
Haemoculture-based	Negative	31	18
	Positive	4	4
	Total	**35**	**22**

738 **Table 3** A) Percentage of patient per CRP-based group. B) Distribution of CSF and plasma samples across CRP- and
739 haemoculture-based groups. CRP groups were defined as follows: CRP group 1 = negative CRP at onset and control (12–
740 18 h); CRP group 2 = negative at onset, positive at control; CRP group 3 = positive at both timepoints. Haemoculture
741 groups were defined by negative or positive microbiological culture results. Values indicate the number of CSF and
742 plasma samples available for proteomic analysis.

743

744 3.2 Proteomic profiling of CSF and plasma

745 Proteomic analysis was performed on 35 CSF and 22 plasma samples (Table 1). In total, 1748 proteins
746 were identified in the CSF, with an average of 1190 proteins per sample, and 1515 in the plasma
747 compartment, with an average of 847 proteins per sample (Fig. 1a). The abundance profiles spanned
748 a wide dynamic range, from highly represented plasma proteins such as albumin, apolipoproteins,
749 transferrin, and haptoglobin to lower-abundance but biologically relevant markers including acute-
750 phase proteins (CRP, LRG1, SAA1/2), adhesion molecules (VCAM1, ICAM1), and the lectin
751 pathway protease MASP1. In CSF, alongside plasma-derived proteins (albumin, immunoglobulins),
752 the dataset encompassed classical CSF markers (cystatin C, transthyretin) and neuronal proteins
753 (APP, NCAM1, IGLON5, CADM1/2, GAP43), reflecting both systemic and CNS-specific
754 contributions (Fig. 1b). Overall, 571 proteins were consistently detected in both compartments,
755 whereas 557 were unique to CSF and 183 to plasma.

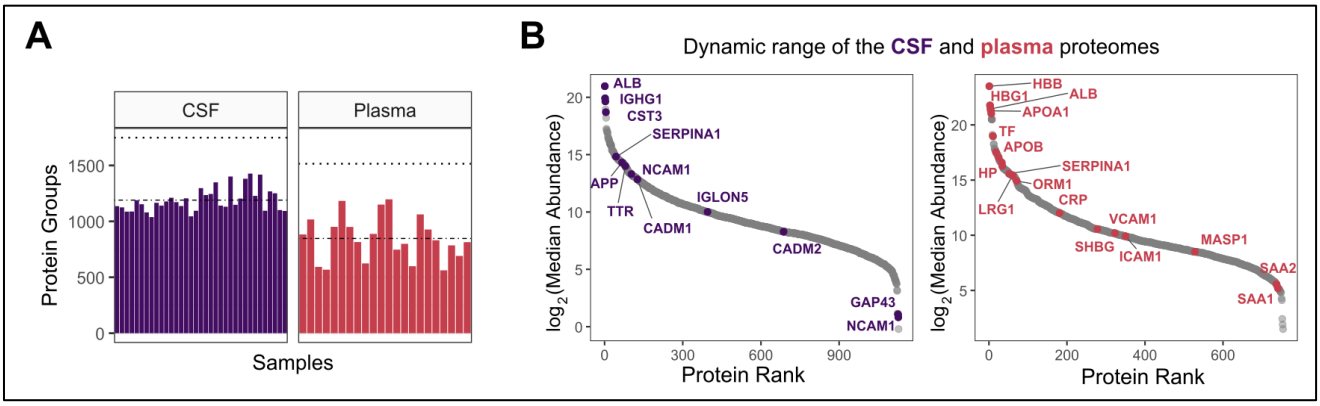


FIGURE 1 Overview of the CSF and plasma proteomes. (a) Total number of proteins identified in each CSF and plasma sample. Dotted lines indicate the global number of protein groups identified in each dataset, while dashed lines indicate the mean number of proteins per sample. (b) Dynamic range of the CSF and plasma proteomes. Proteins are ranked by their median abundance (log_2 scale), highlighting both highly abundant proteins of systemic origin (e.g., ALB, APOA1, TF SERPINA1) and compartment-specific or lower-abundance proteins (e.g., CST3, TTR, APP, GAP43 in CSF; MASP1, VCAM1, SAA1/2 in plasma). This representation illustrates the broad coverage achieved by mass spectrometry and the distinct biological features of each compartment.

3.3 Comparative analysis of CRP-based groups

To investigate the impact of systemic inflammation on the neonatal proteome, patients were stratified into three subgroups based on the temporal dynamics of serum CRP, a routinely used clinical biomarker of systemic inflammation in neonates (Table 3). Group 1 comprised infants with persistently negative CRP values at both onset of symptoms and the 12-18 h control; group 2 included those who were initially CRP-negative but became positive at control; and group 3 consisted of infants with positive CRP values at both timepoints.

After applying quality control (proteins retained if quantified in ≥ 70% of samples in at least one group), 1128 proteins in CSF and 754 in plasma datasets were retained for statistical analysis. One-way ANOVA identified 57 differentially expressed proteins (DEPs) in CSF and 20 in plasma, with four proteins consistently modulated in both compartments: C-reactive protein (CRP), complement component 9 (C9), leucine-rich alpha-2-glycoprotein 1 (LRG1), and alpha-fetoprotein (AFP) (Fig. 2a-2b).

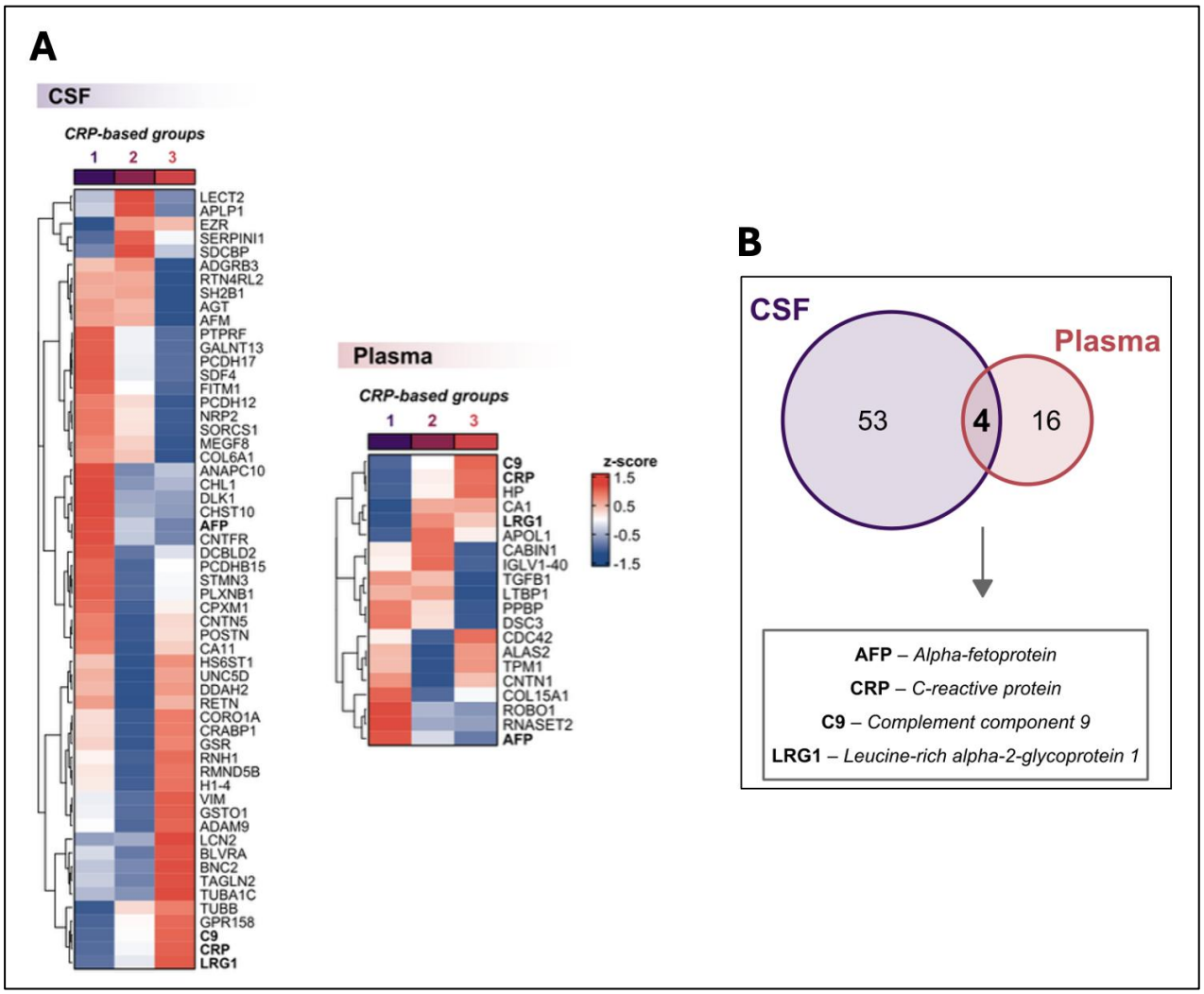


Figure 2. **Shared and compartment-specific proteomic changes across CRP groups.** (a) Heatmaps of DEPs in CSF (left) and plasma (right) across CRP-based groups. Values represent z-scored group medians of protein abundances, and hierarchical clustering was applied across proteins. Four proteins (CRP, C9, LRG1, AFP) were consistently regulated in both compartments (highlighted in bold). (b) Venn diagram showing the overlap of DEPs in CSF (n = 57) and plasma (n = 20). Only four proteins were shared, and their full names are reported in the box.

CRP, C9, and LRG1 showed a progressive increase across CRP groups, whereas AFP showed the opposite trend, with lower levels in inflamed compared with non-inflamed states (Fig. 3).

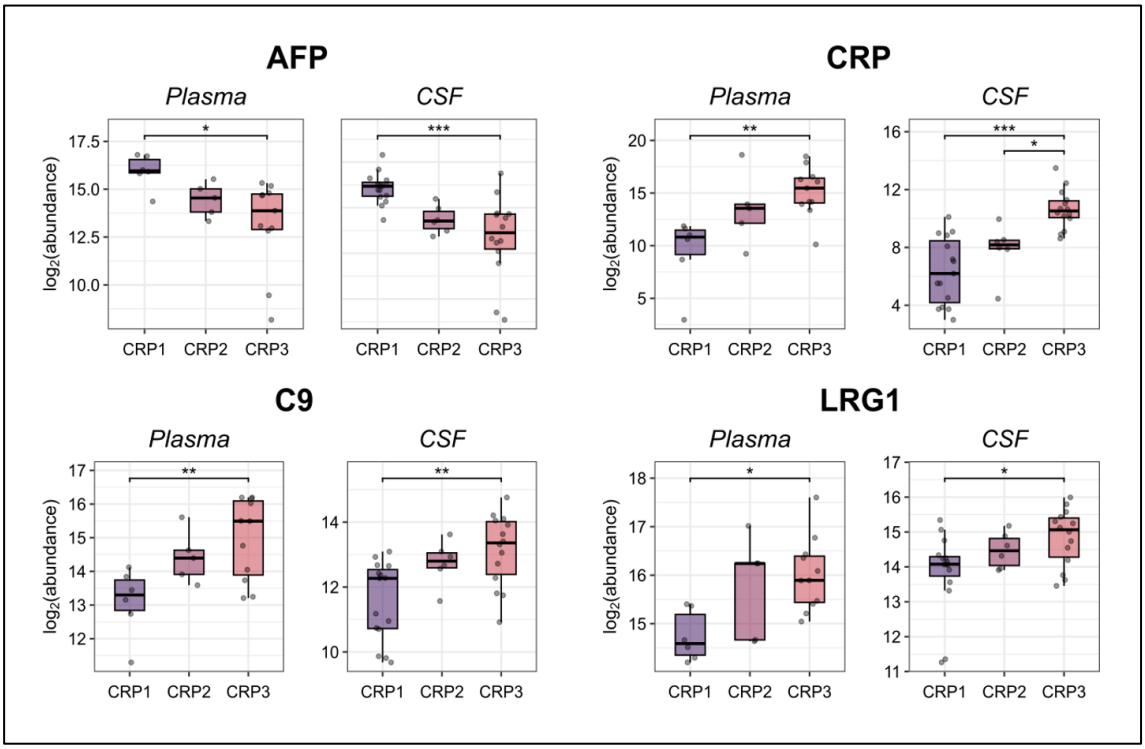


Figure 3. Boxplots of the four shared proteins (AFP, CRP, C9, LRG1) in plasma and CSF across CRP groups. Data are shown as log2-transformed abundances. Boxes represent the interquartile range, horizontal lines indicate the median, and whiskers extend to 1.5× the interquartile range. p-values were obtained using one-way ANOVA followed by Tukey's post-hoc test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Although informative, the plasma proteome captured only a limited number of changes, largely reflecting classical acute-phase mediators (CRP, C9, LRG1, HP) together with AFP and a few structural or adhesion-related proteins (e.g., CNTN1, DSC3, ROBO1). By contrast, the CSF proteome showed a broader and more heterogeneous modulation. In addition to the inflammatory mediators also detected in plasma, CSF-specific DEPs included proteins involved in cell adhesion and axon guidance (CNTN5, CHL1, UNC5D, PCDH17, PLXNB1, PCDH12, PCDHB15), cytoskeletal dynamics (STMN3, TUBA1C, TUBB, VIM, EZR, TAGLN2), and neuronal signalling (APLP1, CNTFR, GPR158, SORCS1). Several stress- and metabolism-associated proteins, including GSR, GSTO1, BLVRA, and LCN2, were also significantly modulated.

To capture the functional meaning of changes observed in the CSF proteome, we performed gene set enrichment analysis (GSEA) using the Gene Ontology Biological Process database (GO BP). This analysis revealed a negative enrichment of pathways related to neurogenesis, neuronal differentiation, cell–cell adhesion, and axon guidance, whereas processes associated with cytoskeleton organization and actin filament remodelling showed positive enrichment (Fig. 4a-b).

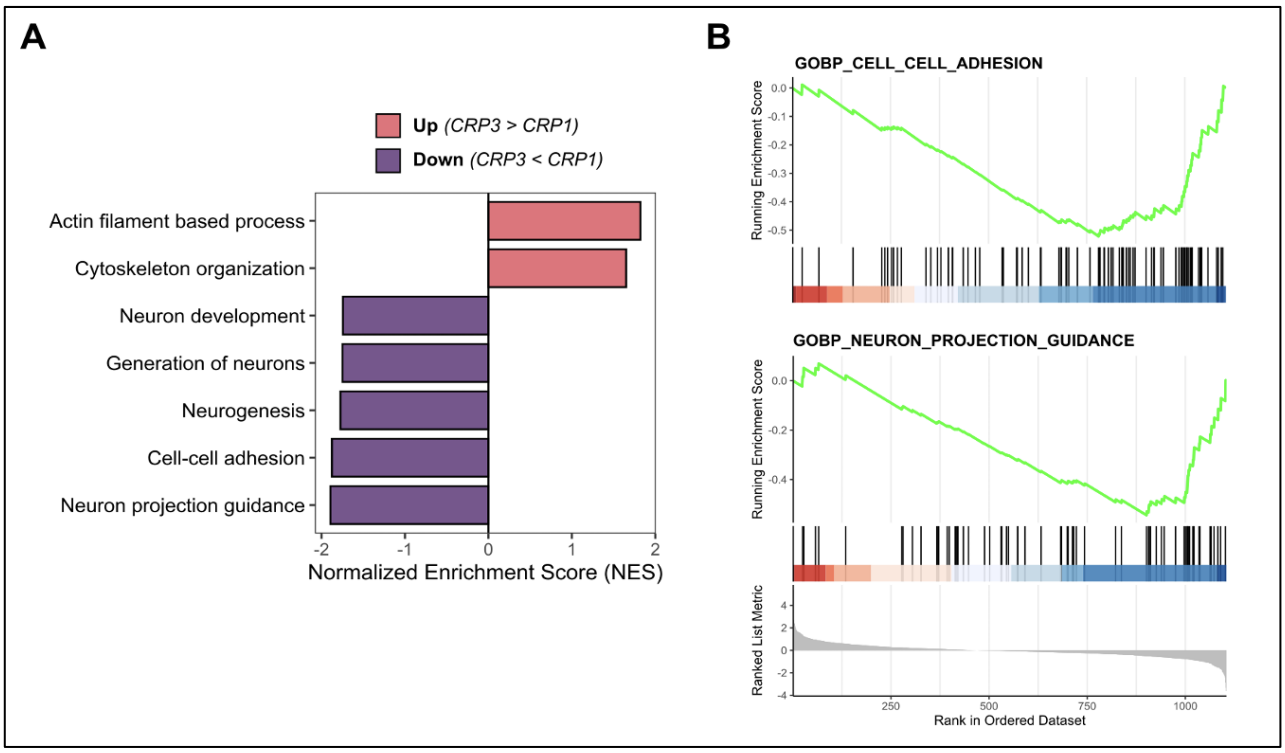


Figure 4. Functional enrichment analysis of CSF proteomic changes across CRP groups. (a) Gene set enrichment analysis (GSEA) of proteins identified in CSF, ranked by signed (CRP3 vs CRP1) –log10 ANOVA p-value. Barplot shows the top significantly enriched up-regulated (red) and down-regulated (purple) gene ontology biological process (go bp) terms. (b) Representative enrichment plots are shown for cell–cell adhesion (top) and neuron projection guidance (bottom), illustrating the distribution of proteins within the ranked list and their contribution to the enrichment score (green curve).

Finally, we examined the connectivity of DEPs through a protein-protein interaction (PPI) network generated with the stringApp database and visualized in Cytoscape. This analysis revealed that modulated proteins clustered into coherent modules, with inflammatory mediators at central position. The functional enrichment provided by stringApp confirmed these patterns, recovering the same biological categories identified by GSEA (Fig. 5).

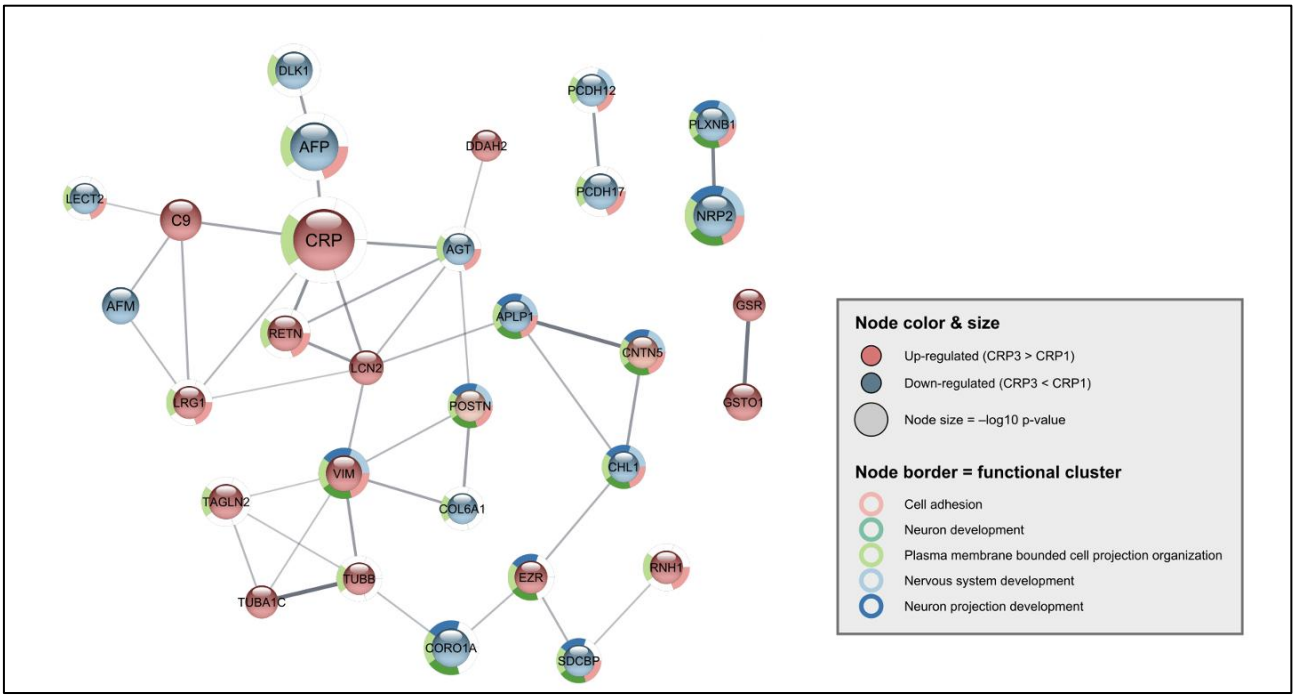


Figure 5. Protein–protein interaction (PPI) network generated in Cytoscape with stringApp using CSF DEPs. Node colours indicate direction of regulation (red = up-regulated in CRP3 vs CRP1, blue = down-regulated), node size reflects statistical significance (–log10 ANOVA p-value), and node borders represent membership in significantly enriched go bp clusters (FDR < 0.01). Edges represent known or predicted protein-protein interactions from stringApp.

Taken together, these complementary approaches demonstrate that the CSF proteome not only mirrors systemic inflammation through the modulation of acute phase proteins (APPs) but also reveals a coordinated regulation of neurodevelopmental and structural organization modules.

3.4 Comparative analysis of haemoculture-based groups

To investigate whether the neonatal proteome reflects the presence of microbiologically confirmed sepsis, we compared samples from patients with positive versus negative haemocultures (Table 1). This analysis was performed separately in CSF and plasma to evaluate whether pathogen isolation was associated with specific proteomic signatures beyond those captured by CRP dynamics.

After filtering proteins as previously described, 1101 proteins in CSF and 746 in plasma were retained for statistical testing. Group sizes were highly unbalanced (31 vs 4 CSF samples; 18 vs 4 plasma

samples), which inevitably reduced statistical power. Within this context, differential analysis using a two-sided Student's t-test (s_0 = 0.1 and permutation-based FDR < 0.05) identified only one significantly modulated protein: mannan-binding lectin serine protease 1 (MASP1), which was down-regulated in the CSF of culture-positive infants ($\log_2$ fold change = –3.2; q-value = 0.02). No significant changes were detected in plasma. MASP1 is a serine protease that functions as a component of the lectin pathway of complement activation and is also able to cleave fibrinogen and factor XIII, thus potentially being involved in coagulation.

4. Discussion

Systemic inflammation in early life has been increasingly recognised as a critical modifier of CNS development, yet the behaviour of the neonatal blood-CSF barrier under these conditions remains poorly defined.

Clinical and demographic characteristics were largely comparable across CRP-defined groups (Tables 1–2), with the notable exception that infants in CRP group 1 were significantly more immature at the time of LOS suspicion. This immaturity was reflected by a lower corrected gestational age, lower 1-minute Apgar scores, and earlier gestational age at antenatal steroid administration, consistent with the greater diagnostic challenge of LOS in very preterm infants. Importantly, by study design, infants with meningitis—defined by positive CSF culture or elevated CSF cell counts—were excluded, allowing the observed CSF proteomic differences across inflammatory stages to be interpreted in the absence of overt CNS infection and to be attributed to inflammation-related modulation of blood-CSF barrier function rather than major clinical confounders.

The present study aimed to characterize the behaviour of the neonatal blood-CSF barrier during systemic inflammation, with the goal of elucidating mechanisms underlying neurological

involvement occurring after LOS, particularly in VLBW preterm infants, even in absence of meningitis [107], [108], [109], [110], [111]. The issue is clinically relevant, as inflammatory cytokine profiles have been repeatedly associated with adverse neurodevelopmental outcomes in preterm infants [112], and neurodevelopmental impairments persist despite advancements in neonatal care. Understanding how systemic inflammation interacts with CNS compartments is therefore essential to develop targeted neuroprotective strategies. [113], [114]

Using global proteomic profiling of temporally matched CSF and plasma samples, our study provides a comprehensive overview of how systemic inflammation affects distinct biological compartments in neonates. Notably, CSF exhibited greater proteomic complexity than plasma, both in terms of the total number of identified proteins and the average number of proteins per sample. While a substantial proportion of proteins was shared between CSF and plasma, many proteins were uniquely identified in the CSF, including well-established CSF markers as well as proteins implicated in neuronal and synaptic functions. [69], [115] These findings reinforce the concept that CSF proteome should not be viewed merely as a byproduct of plasma filtration but represents a complex biologically active compartment shaped by systemic and CNS-specific processes. [116]

The stark disparity in protein abundance observed across compartments -from highly abundant plasma-derived proteins (e.g., ALB, APOA1, TF, SERPINA1) to low-abundance but biologically relevant inflammatory and neuronal markers (e.g., CST3, TTR, APP, GAP43 in CSF; MASP1, VCAM1, SAA1/2 in plasma)- emphasizes the utility of proteomic techniques in capturing compartment-specific responses to systemic inflammation. [115], [116] The identification of CSF-exclusive proteins provides a strong biological rationale for focusing on CSF as a privileged medium for investigating and detecting neurobiological changes linked to LOS. Collectively, these findings support the hypothesis that systemic inflammation induces distinct molecular signatures within the CNS, potentially enabling early identification of neurobiological vulnerability in neonates. [6], [117]

To stratify systemic inflammation, we adopted a CRP trend-based approach rather than relying on isolated CRP values, which can be influenced by transient, non-infectious factors. This strategy was supported by the concordance of routinary plasmatic immunoassay-based measurement and CRP detected in both plasma and CSF by MS. The presence of a clear proteomic gradient across CRP groups further validates this approach, suggesting that CRP dynamics reflect biologically distinct stages of systemic inflammation rather than a simple dichotomous state.

The temporally matched CSF and plasma sampling was a major strength of this study, allowing direct assessment of shared and compartment-specific protein modulation and providing *in vivo* insights into neonatal blood-CSF barrier dynamics. Across CRP dynamics-defined stages of inflammation, despite similar demographics of the three groups, we identified significant modulation of 57 CSF and 20 plasma proteins, with a markedly broader and more diverse response in CSF. This disproportionate CNS response suggests that systemic inflammation is not merely transmitted passively to the CNS but is amplified and biologically diversified within the CSF compartment. Several acute-phase proteins (APPs), including CRP, LRG1, and C9, showed concordant modulation in both compartments with advancing inflammation, providing direct insights into blood-CSF barrier dynamics [118]. Notably, the markedly higher number and diversity of differentially expressed proteins detected in the CSF compared to plasma suggest that systemic inflammation is amplified and biologically diversified within the CNS compartment.

Our main objective was to explore whether inflammatory protein changes in CSF could help explain the neurological impairment often associated with LOS in this fragile population. We focused on LOS as one of the most frequent causes of systemic inflammation in NICU patients; none of the enrolled infants fulfilled diagnostic criteria for meningitis. A total of 45 temporally matched pairs of CSF and plasma samples collected during evaluation for suspected LOS were analysed by high-resolution MS. This paired design enabled direct assessment of shared and compartment-specific proteomic changes,

offering a unique opportunity to investigate blood-CSF barrier dynamics *in vivo* in a clinical neonatal setting.

Because albumin is exclusively synthesised in the liver and not in the CNS [119], [120], the CSF/serum albumin ratio remains a classical indicator of blood-CSF barrier integrity. In our study, APPs such as CRP, LRG1, and C9 showed parallel increase in plasma and CSF across different stages of inflammation, suggesting translocation from the bloodstream to the CSF via increased permeability of the blood-CSF barrier during LOS. Importantly, the absence of significant changes in the CSF/serum albumin ratio supports the interpretation that these findings reflect selective and regulated permeability changes, rather than gross structural disruption of the blood-CSF barrier (or of the BBB more broadly). This pattern is consistent with a functional modulation of the blood-CSF barrier, permitting controlled passage of specific inflammatory mediators while preserving overall integrity, rather than non-selective leakage. Nevertheless, technical and biological factors – including circadian variation, CSF sampling volume, and rostro-caudal gradients [38]– may influence CSF protein concentrations and must be considered when interpreting these data.

Proteomic analysis revealed significant down-regulation of MASP1 in the CSF of haemoculture-positive infants. Reduced MASP-1 concentrations are associated with impaired coagulation in septic shock patients [121], [122], suggesting that increased MASP-1 activation and consumption may also contribute to coagulation disturbances during neonatal sepsis. This finding points to a potential role for MASP-1 in sepsis-associated disseminated intravascular coagulation (DIC). However, because our study did not include a systematic analysis of coagulation parameters, we cannot determine whether MASP1 downregulation in CSF correlates with significant coagulopathy. Further studies on a larger population are needed to clarify this aspect. The selective detection of MASP1 modulation in the CSF, but not in plasma, further supports the concept that inflammatory and coagulation-related processes may be differentially regulated within the CNS compartment during confirmed sepsis.

Beyond APPs translocation and MASP-1 consumption, we observed a broader stage-dependent modulation of the CSF proteome. Functional enrichment analyses showed increasing representation of processes related to cytoskeleton organization and actin filament dynamics with advancing inflammation, whereas processes linked to neuronal development, axon guidance, and cell-cell adhesion were more prominent at lower inflammatory stages. These findings suggest a transition from a neurodevelopmental molecular profile towards a stress- and structure-oriented response as systemic inflammation progresses, potentially reflecting an adaptive - but developmentally costly- reprogramming of the CNS environment. Lower stages of systemic inflammation appear to preserve neuronal integrity while maintaining molecular processes involved in CNS migration and maturation, whereas higher inflammatory correlate to cytoskeletal reorganization, consistent with increased blood-CSF barrier permeability. Previous studies have shown that altered BBB permeability in response to systemic inflammation can induce structural and functional changes in CSF composition [123], [124], aligning with our findings. In this context, cytoskeletal remodelling may represent both a marker and a mediator of barrier adaptation, with potential downstream effects on neuronal connectivity and maturation. The actin filament dynamics observed could reflect the inflammatory-driven network reconfiguration aimed at for inflammation clearance, corroborating earlier reports of similar cytoskeletal adaptations in early onset (<72 h of life) neuroinflammatory conditions in a smaller cohort of patients. [125]

Protein-protein interaction analyses further demonstrated that modulated proteins cluster into coordinated functional networks, reinforcing the concept that systemic inflammation in LOS is mirrored by pathway-level alteration in the CSF proteome rather than isolated protein changes.

Altogether, these findings support the notion that the CSF proteome functions as an integrated sensor of systemic inflammation and its neurobiological consequences, highlighting the blood-CSF barrier as a dynamic interface potentially involved in shaping long-term neurodevelopmental outcomes in neonates with LOS.

5. Conclusions

The present thesis was designed to investigate how systemic inflammation during neonatal LOS interacts with CNS compartments, focusing on proteomic modulation in plasma and CSF, potential mechanisms of neurological involvement, and the functional state of the blood-CSF barrier.

First, by analysing temporally matched plasma and CSF samples from term and preterm infants across different stages of systemic inflammation, this work provides *in vivo* evidence of compartment-specific proteomic modulation. This suggests that systemic inflammation in not merely transmitted passively to the CNS but is amplified and biologically diversified within the CSF compartment.
The integration of proteomic data with CRP levels supports the use of CRP as a quantitative marker of inflammatory burden, while also highlighting its biological relevance in relation to protein changes occurring within the CSF compartment.

Second, the identification of inflammation-associated proteomic signatures in CSF, in the absence of biochemical or microbiological evidence of CNS infection, suggest that neurological involvement during LOS may occur through indirect inflammatory mechanisms. In particular, the inflammatory stage-dependent modulation of CSF proteins and pathways related to neurogenesis, neuron projection guidance, and synaptic function provides insight into potential biological processes linking systemic inflammation to subclinical CNS involvement.

Third, the limited overlap between plasma and CSF proteomic changes, together with the absence of classical marker of barrier breakdown, supports a model of functional modulation rather than structural disruption of the blood-CSF barrier during neonatal LOS. These findings provide *in vivo* evidence that the neonatal blood-CSF barrier is developmentally competent and dynamically regulated during inflammatory stress.

Taken together, the results of this thesis contribute to a more integrated understanding of neonatal neuroinflammation, brain barrier physiology, and peripheral-central inflammatory crosstalk during LOS in newborns. By clarifying the biological meaning of proteomic changes in CSF and plasma, and their relationship with systemic inflammation, this work lays the groundwork for future studies aimed at identifying early biomarkers of CNS involvement and potential targets for neuroprotective strategies in vulnerable newborn.

References

[1] E. Horváth-Puhó *et al.*, "Mortality, neurodevelopmental impairments, and economic outcomes after invasive group B streptococcal disease in early infancy in Denmark and the Netherlands: a national matched cohort study," *Lancet Child Adolesc. Health*, vol. 5, no. 6, pp. 398–407, Jun. 2021, doi: 10.1016/S2352-4642(21)00022-5.

[2] L. J. Schlapbach *et al.*, "Impact of sepsis on neurodevelopmental outcome in a Swiss National Cohort of extremely premature infants," *Pediatrics*, vol. 128, no. 2, Aug. 2011, doi: 10.1542/PEDS.2010-3338.

[3] T. Strunk, T. Inder, X. Wang, D. Burgner, C. Mallard, and O. Levy, "Infection-induced inflammation and cerebral injury in preterm infants," *Lancet Infect. Dis.*, vol. 14, no. 8, pp. 751–762, 2014, doi: 10.1016/S1473-3099(14)70710-8.

[4] Q. Gao and M. S. Hernandes, "Sepsis-Associated Encephalopathy and Blood-Brain Barrier Dysfunction," *Inflammation*, vol. 44, no. 6, pp. 2143–2150, Dec. 2021, doi: 10.1007/S10753-021-01501-3.

[5] T. Muk *et al.*, “Rapid Proteome Changes in Plasma and Cerebrospinal Fluid Following Bacterial Infection in Preterm Newborn Pigs,” *Front. Immunol.*, vol. 10, Nov. 2019, doi: 10.3389/FIMMU.2019.02651.

[6] P. P. Jiang, S. S. Peng, S. Pankratova, P. Luo, P. Zhou, and Y. Chen, “Proteins Involved in Synaptic Plasticity Are Downregulated in the Cerebrospinal Fluid of Infants With Clinical Sepsis Complicated by Neuroinflammation,” *Front. Cell. Neurosci.*, vol. 16, May 2022, doi: 10.3389/FNCEL.2022.887212.

[7] N. Sekino, M. Selim, and A. Shehadah, “Sepsis-associated brain injury: underlying mechanisms and potential therapeutic strategies for acute and long-term cognitive impairments,” *J. Neuroinflammation*, vol. 19, no. 1, Dec. 2022, doi: 10.1186/S12974-022-02464-4.

[8] G. A. Dunn, J. T. Nigg, and E. L. Sullivan, “Neuroinflammation as a risk factor for attention deficit hyperactivity disorder,” *Pharmacol. Biochem. Behav.*, vol. 182, pp. 22–34, Jul. 2019, doi: 10.1016/J.PBB.2019.05.005.

[9] T. Barichello, G. D. Fagundes, J. S. Generoso, S. G. Elias, L. R. Simões, and A. L. Teixeira, “Pathophysiology of neonatal acute bacterial meningitis,” *J. Med. Microbiol.*, vol. 62, no. Pt 12, pp. 1781–1789, Dec. 2013, doi: 10.1099/JMM.0.059840-0.

[10] E. C. B. Johnson *et al.*, “Large-scale proteomic analysis of Alzheimer’s disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation,” *Nat. Med.*, vol. 26, no. 5, pp. 769–780, May 2020, doi: 10.1038/S41591-020-0815-6.

[11] M. Saade, G. Araujo de Souza, C. Scavone, and P. F. Kinoshita, “The Role of GPNMB in Inflammation,” *Front. Immunol.*, vol. 12, May 2021, doi: 10.3389/FIMMU.2021.674739.

[12] L. Stridh *et al.*, “Toll-like receptor-3 activation increases the vulnerability of the neonatal brain to hypoxia-ischemia,” *J. Neurosci.*, vol. 33, no. 29, pp. 12041–12051, 2013, doi: 10.1523/JNEUROSCI.0673-13.2013.

[13] J. J. Iliff *et al.*, “A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β,” *Sci. Transl. Med.*, vol. 4, no. 147, Aug. 2012, doi: 10.1126/SCITRANSLMED.3003748.

[14] T. M. O’Shea *et al.*, “Elevated blood levels of inflammation-related proteins are associated with an attention problem at age 24 mo in extremely preterm infants,” *Pediatr. Res.*, vol. 75, no. 6, pp. 781–787, 2014, doi: 10.1038/PR.2014.41.

[15] A. Mottahedin, P. L. P. Smith, H. Hagberg, C. J. Ek, and C. Mallard, “TLR2-mediated leukocyte trafficking to the developing brain,” *J. Leukoc. Biol.*, vol. 101, no. 1, pp. 297–305, Jan. 2017, doi: 10.1189/JLB.3A1215-568R.

[16] A. Rayasam, Y. Fukuzaki, and Z. S. Vexler, “Microglia-leucocyte axis in cerebral ischaemia and inflammation in the developing brain,” *Acta Physiol. (Oxf).*, vol. 233, no. 1, Sep. 2021, doi: 10.1111/APHA.13674.

[17] S. Gyoneva *et al.*, “Systemic inflammation regulates microglial responses to tissue damage in vivo,” *Glia*, vol. 62, no. 8, pp. 1345–1360, 2014, doi: 10.1002/GLIA.22686.

[18] L. Liu *et al.*, “Single-nucleus transcriptomics reveals sepsis-related neurovascular dysfunction in the human hippocampus,” *Front. Immunol.*, vol. 16, 2025, doi: 10.3389/FIMMU.2025.1648278.

[19] R. Daneman and A. Prat, “The blood-brain barrier,” *Cold Spring Harb. Perspect. Biol.*, vol. 7, no. 1, Jan. 2015, doi: 10.1101/CSHPERSPECT.A020412.

[20] E. C. B. Johnson *et al.*, "Large-scale proteomic analysis of Alzheimer's disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation," *Nat. Med.*, vol. 26, no. 5, pp. 769–780, May 2020, doi: 10.1038/S41591-020-0815-6.

[21] J. M. Hengst, "The role of C-reactive protein in the evaluation and management of infants with suspected sepsis," *Adv. Neonatal Care*, vol. 3, no. 1, pp. 3–13, Feb. 2003, doi: 10.1053/ADNC.2003.50010.

[22] N. Hofer, E. Zacharias, W. Müller, and B. Resch, "An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks," *Neonatology*, vol. 102, no. 1, pp. 25–36, 2012, doi: 10.1159/000336629.

[23] M. B. Pepys and G. M. Hirschfield, "C-reactive protein: A critical update," *Journal of Clinical Investigation*, vol. 111, no. 12, pp. 1805–1812, Jun. 2003, doi: 10.1172/JCI200318921.

[24] K. R. Ludwig and A. B. Hummon, "Mass spectrometry for the discovery of biomarkers of sepsis," *Mol. Biosyst.*, vol. 13, no. 4, pp. 648–664, 2017, doi: 10.1039/c6mb00656f.

[25] J. J. Alexander, A. Jacob, P. Cunningham, L. Hensley, and R. J. Quigg, "TNF is a key mediator of septic encephalopathy acting through its receptor, TNF receptor-1," *Neurochem. Int.*, vol. 52, no. 3, pp. 447–456, Feb. 2008, doi: 10.1016/J.NEUINT.2007.08.006.

[26] S. M. Gordon, L. Srinivasan, and M. C. Harris, "Neonatal Meningitis: Overcoming Challenges in Diagnosis, Prognosis, and Treatment with Omics," *Front. Pediatr.*, vol. 5, Jun. 2017, doi: 10.3389/FPED.2017.00139.

[27] E. M. Graham, A. D. Everett, J. C. Delpech, and F. J. Northington, "Blood biomarkers for evaluation of perinatal encephalopathy: State of the art," *Curr. Opin. Pediatr.*, vol. 30, no. 2, pp. 199–203, Apr. 2018, doi: 10.1097/MOP.0000000000000591.

[28] S. McGrane *et al.*, “Procalcitonin and C-reactive protein levels at admission as predictors of duration of acute brain dysfunction in critically ill patients,” *Crit. Care*, vol. 15, no. 2, Mar. 2011, doi: 10.1186/CC10070.

[29] M. D. Nist and R. H. Pickler, “An Integrative Review of Cytokine/Chemokine Predictors of Neurodevelopment in Preterm Infants,” *Biol. Res. Nurs.*, vol. 21, no. 4, pp. 366–376, Jul. 2019, doi: 10.1177/1099800419852766.

[30] G. Biouss *et al.*, “Experimental necrotizing enterocolitis induces neuroinflammation in the neonatal brain,” *J. Neuroinflammation*, vol. 16, no. 1, May 2019, doi: 10.1186/s12974-019-1481-9.

[31] B. J. Stoll *et al.*, “Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection,” *JAMA*, vol. 292, no. 19, pp. 2357–2365, Nov. 2004, doi: 10.1001/JAMA.292.19.2357.

[32] L. Gaetani *et al.*, “CSF and Blood Biomarkers in Neuroinflammatory and Neurodegenerative Diseases: Implications for Treatment,” *Trends Pharmacol. Sci.*, vol. 41, no. 12, pp. 1023–1037, Dec. 2020, doi: 10.1016/J.TIPS.2020.09.011,.

[33] N. R. Saunders, K. M. Dziegielewska, K. Møllgård, and M. D. Habgood, “Physiology and molecular biology of barrier mechanisms in the fetal and neonatal brain,” *J. Physiol.*, vol. 596, no. 23, pp. 5723–5756, Dec. 2018, doi: 10.1113/JP275376.

[34] X. Du *et al.*, “Systemic stimulation of TLR2 impairs neonatal mouse brain development,” *PLoS One*, vol. 6, no. 5, 2011, doi: 10.1371/JOURNAL.PONE.0019583.

[35] N. S. Groeneveld *et al.*, “Cerebrospinal fluid inflammatory markers to differentiate between neonatal bacterial meningitis and sepsis: A prospective study of diagnostic accuracy,”

International Journal of Infectious Diseases, vol. 142, May 2024, doi: 10.1016/J.IJID.2024.02.013,.

[36] S. H. Shin, E. K. Kim, K. Y. Lee, and H. S. Kim, "TNF-α antagonist attenuates systemic lipopolysaccharide-induced brain white matter injury in neonatal rats," *BMC Neurosci.*, vol. 20, no. 1, Aug. 2019, doi: 10.1186/S12868-019-0529-1.

[37] S. Shu *et al.*, "Hypoxia and hypoxia-inducible factors in kidney injury and repair," *Cells*, vol. 8, no. 3, Mar. 2019, doi: 10.3390/cells8030207.

[38] B. Fleiss, J. Van Steenwinckel, C. Bokobza, I. K. Shearer, E. Ross-Munro, and P. Gressens, "Microglia-Mediated Neurodegeneration in Perinatal Brain Injuries," *Biomolecules*, vol. 11, no. 1, pp. 1–22, Jan. 2021, doi: 10.3390/BIOM11010099.

[39] D. F. Niño *et al.*, "Cognitive impairments induced by necrotizing enterocolitis can be prevented by inhibiting microglial activation in mouse brain," *Sci. Transl. Med.*, vol. 10, no. 471, Dec. 2018, doi: 10.1126/SCITRANSLMED.AAN0237.

[40] L. G. Costa, T. B. Cole, J. Coburn, Y. C. Chang, K. Dao, and P. J. Roqué, "Neurotoxicity of traffic-related air pollution," *Neurotoxicology*, vol. 59, pp. 133–139, Mar. 2017, doi: 10.1016/j.neuro.2015.11.008.

[41] N. R. Saunders, K. M. Dziegielewska, K. Møllgård, and M. D. Habgood, "Physiology and molecular biology of barrier mechanisms in the fetal and neonatal brain," *J. Physiol.*, vol. 596, no. 23, pp. 5723–5756, Dec. 2018, doi: 10.1113/JP275376.

[42] M. W. Brightman and T. S. Reese, "Junctions between intimately apposed cell membranes in the vertebrate brain," *J. Cell Biol.*, vol. 40, no. 3, pp. 648–677, 1969, doi: 10.1083/JCB.40.3.648.

[43] B. Engelhardt and L. Sorokin, “The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction,” *Semin. Immunopathol.*, vol. 31, no. 4, pp. 497–511, Nov. 2009, doi: 10.1007/S00281-009-0177-0.

[44] N. J. Abbott, L. Rönnbäck, and E. Hansson, “Astrocyte-endothelial interactions at the blood-brain barrier,” *Nat. Rev. Neurosci.*, vol. 7, no. 1, pp. 41–53, Jan. 2006, doi: 10.1038/NRN1824.

[45] P. A. Johansson, K. M. Dziegielewska, S. A. Liddelow, and N. R. Saunders, “The blood-CSF barrier explained: when development is not immaturity,” *Bioessays*, vol. 30, no. 3, pp. 237–248, Mar. 2008, doi: 10.1002/BIES.20718.

[46] K. Møllgård, Y. Balslev, B. Lauritzen, and N. R. Saunders, “Cell junctions and membrane specializations in the ventricular zone (germinal matrix) of the developing sheep brain: a CSF-brain barrier,” *J. Neurocytol.*, vol. 16, no. 4, pp. 433–444, Aug. 1987, doi: 10.1007/BF01668498.

[47] J. K. Madsen and K. Møllgård, “The tight epithelium of the Mongolian gerbil subcommissural organ as revealed by freeze-fracturing,” *J. Neurocytol.*, vol. 8, no. 4, pp. 481–491, Aug. 1979, doi: 10.1007/BF01214804.

[48] K. Møllgård, Y. Balslev, B. Lauritzen, and N. R. Saunders, “Cell junctions and membrane specializations in the ventricular zone (germinal matrix) of the developing sheep brain: a CSF-brain barrier,” *J. Neurocytol.*, vol. 16, no. 4, pp. 433–444, Aug. 1987, doi: 10.1007/BF01668498.

[49] G. Fossan *et al.*, “CSF-brain permeability in the immature sheep fetus: a CSF-brain barrier,” *Brain Res.*, vol. 350, no. 1–2, pp. 113–124, 1985, doi: 10.1016/0165-3806(85)90255-X.

[50] S. Whish *et al.*, “The inner CSF-brain barrier: developmentally controlled access to the brain via intercellular junctions,” *Front. Neurosci.*, vol. 9, no. FEB, 2015, doi: 10.3389/FNINS.2015.00016.

[51] N. R. Saunders, S. A. Liddelow, and K. M. Dziegielewska, “Barrier mechanisms in the developing brain,” *Front. Pharmacol.*, vol. 3, pp. 1–18, 2012, doi: 10.3389/FPHAR.2012.00046.

[52] O. Dammann and A. Leviton, “Intermittent or sustained systemic inflammation and the preterm brain,” *Pediatr. Res.*, vol. 75, no. 3, pp. 376–380, Mar. 2014, doi: 10.1038/pr.2013.238.

[53] S. Gazzin *et al.*, “Differential expression of the multidrug resistance-related proteins ABCb1 and ABCc1 between blood-brain interfaces,” *J. Comp. Neurol.*, vol. 510, no. 5, pp. 497–507, Oct. 2008, doi: 10.1002/CNE.21808.

[54] M. Daood, C. Tsai, M. Ahdab-Barmada, and J. F. Watchko, “ABC transporter (P-gp/ABCB1, MRP1/ABCC1, BCRP/ABCG2) expression in the developing human CNS,” *Neuropediatrics*, vol. 39, no. 4, pp. 211–218, Aug. 2008, doi: 10.1055/S-0028-1103272.

[55] I. Kratzer, S. A. Liddelow, N. R. Saunders, K. M. Dziegielewska, N. Strazielle, and J. F. Ghersi-Egea, “Developmental changes in the transcriptome of the rat choroid plexus in relation to neuroprotection,” *Fluids Barriers CNS*, vol. 10, no. 1, Aug. 2013, doi: 10.1186/2045-8118-10-25.

[56] S. Tietz and B. Engelhardt, “Brain barriers: Crosstalk between complex tight junctions and adherens junctions,” *J. Cell Biol.*, vol. 209, no. 4, pp. 493–506, 2015, doi: 10.1083/JCB.201412147.

[57] X. Li *et al.*, “Clinical features and antimicrobial susceptibility profiles of culture-proven neonatal sepsis in a tertiary children’s hospital, 2013 to 2017,” *Medicine*, vol. 98, no. 12, 2019, doi: 10.1097/MD.0000000000014686.

[58] N. Strazielle and J. F. Ghersi-Egea, “Physiology of blood-brain interfaces in relation to brain disposition of small compounds and macromolecules,” *Mol. Pharm.*, vol. 10, no. 5, pp. 1473–1491, May 2013, doi: 10.1021/MP300518E.

[59] J. Oberdick, Y. Ling, M. A. Phelps, M. S. Yudovich, K. Schilling, and W. Sadee, “Preferential Delivery of an Opioid Antagonist to the Fetal Brain in Pregnant Mice,” *J. Pharmacol. Exp. Ther.*, vol. 358, no. 1, pp. 22–30, Jul. 2016, doi: 10.1124/JPET.115.231902.

[60] K. A. Allen, “Is Prenatal Lead Exposure a Concern in Infancy? What Is the Evidence?,” *Adv. Neonatal Care*, vol. 15, no. 6, pp. 416–420, Dec. 2015, doi: 10.1097/ANC.0000000000000224.

[61] M. Amaraneni *et al.*, “Brain uptake of deltamethrin in rats as a function of plasma protein binding and blood-brain barrier maturation,” *Neurotoxicology*, vol. 62, pp. 24–29, Sep. 2017, doi: 10.1016/J.NEURO.2017.04.009.

[62] N. R. Saunders *et al.*, “The rights and wrongs of blood-brain barrier permeability studies: a walk through 100 years of history,” *Front. Neurosci.*, vol. 8, no. DEC, 2014, doi: 10.3389/FNINS.2014.00404.

[63] I. Panfoli *et al.*, “Why do premature newborn infants display elevated blood adenosine levels?,” *Med. Hypotheses*, vol. 90, pp. 53–56, May 2016, doi: 10.1016/J.MEHY.2016.03.007.

[64] Z. Zhao, A. R. Nelson, C. Betsholtz, and B. V. Zlokovic, “Establishment and Dysfunction of the Blood-Brain Barrier,” *Cell*, vol. 163, no. 5, pp. 1064–1078, Nov. 2015, doi: 10.1016/J.CELL.2015.10.067.

[65] H. Shen, D. E. Smith, R. F. Keep, and F. C. Brosius, “Immunolocalization of the proton-coupled oligopeptide transporter PEPT2 in developing rat brain,” *Mol. Pharm.*, vol. 1, no. 4, pp. 248–256, 2004, doi: 10.1021/MP049944B.

[66] O. Dammann and A. Leviton, “Intermittent or sustained systemic inflammation and the preterm brain,” *Pediatr. Res.*, vol. 75, no. 3, pp. 376–380, Mar. 2014, doi: 10.1038/PR.2013.238.

[67] J. Zhao, D. K. Kwang, X. Yang, S. Auh, Y. X. Fu, and H. Tang, “Hyper innate responses in neonates lead to increased morbidity and mortality after infection,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 105, no. 21, pp. 7528–7533, May 2008, doi: 10.1073/PNAS.0800152105.

[68] M. A. Bryniarski, T. Ren, A. R. Rizvi, A. M. Snyder, and M. E. Morris, “Targeting the Choroid Plexuses for Protein Drug Delivery.,” *Pharmaceutics*, vol. 12, no. 10, pp. 1–34, Oct. 2020, doi: 10.3390/pharmaceutics12100963.

[69] P. P. Jiang, S. S. Peng, S. Pankratova, P. Luo, P. Zhou, and Y. Chen, “Proteins Involved in Synaptic Plasticity Are Downregulated in the Cerebrospinal Fluid of Infants With Clinical Sepsis Complicated by Neuroinflammation,” *Front. Cell. Neurosci.*, vol. 16, May 2022, doi: 10.3389/FNCEL.2022.887212.

[70] H. B. Stolp and K. M. Dziegielewska, “Review: Role of developmental inflammation and blood-brain barrier dysfunction in neurodevelopmental and neurodegenerative diseases,” *Neuropathol. Appl. Neurobiol.*, vol. 35, no. 2, pp. 132–146, Apr. 2009, doi: 10.1111/J.1365-2990.2008.01005.X.

[71] H. B. Stolp, K. M. Dziegielewska, C. J. Ek, A. M. Potter, and N. R. Saunders, “Long-term changes in blood-brain barrier permeability and white matter following prolonged systemic inflammation in early development in the rat,” *Eur. J. Neurosci.*, vol. 22, no. 11, pp. 2805–2816, Dec. 2005, doi: 10.1111/J.1460-9568.2005.04483.X.

[72] Y. Ogata, M. C. Charlesworth, and D. C. Muddiman, “Evaluation of protein depletion methods for the analysis of total-, phospho- and glycoproteins in lumbar cerebrospinal fluid,” *J. Proteome Res.*, vol. 4, no. 3, pp. 837–845, May 2005, doi: 10.1021/PR049750O.

[73] R. Goldbach-Mansky *et al.*, “Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition,” *N. Engl. J. Med.*, vol. 355, no. 6, pp. 581–592, Aug. 2006, doi: 10.1056/NEJMOA055137.

[74] C. E. Teunissen *et al.*, “White paper by the Society for CSF Analysis and Clinical Neurochemistry: Overcoming barriers in biomarker development and clinical translation,” *Alzheimers Res. Ther.*, vol. 10, no. 1, Mar. 2018, doi: 10.1186/S13195-018-0359-X.

[75] K. Allegaert, I. Scheers, E. Adams, G. Brajanoski, V. Cossey, and B. J. Anderson, “Cerebrospinal fluid compartmental pharmacokinetics of amikacin in neonates,” *Antimicrob. Agents Chemother.*, vol. 52, no. 6, pp. 1934–1939, Jun. 2008, doi: 10.1128/AAC.01099-07.

[76] E. Fox *et al.*, “The serum and cerebrospinal fluid pharmacokinetics of anakinra after intravenous administration to non-human primates,” *J. Neuroimmunol.*, vol. 223, no. 1–2, pp. 138–140, Jun. 2010, doi: 10.1016/j.jneuroim.2010.03.022.

[77] R. W. Price *et al.*, “Evolving character of chronic central nervous system HIV infection,” *Semin. Neurol.*, vol. 34, no. 1, pp. 7–13, 2014, doi: 10.1055/S-0034-1372337.

[78] B. Battal, M. Kocaoglu, N. Bulakbasi, G. Husmen, H. Tuba Sanal, and C. Tayfun, “Cerebrospinal fluid flow imaging by using phase-contrast MR technique,” *British Journal of Radiology*, vol. 84, no. 1004, pp. 758–765, Aug. 2011, doi: 10.1259/bjr/66206791.

[79] A. Leviton *et al.*, “Neonatal systemic inflammation and the risk of low scores on measures of reading and mathematics achievement at age 10 years among children born extremely

preterm," *Int. J. Dev. Neurosci.*, vol. 66, pp. 45–53, May 2018, doi: 10.1016/J.IJDEVNEU.2018.01.001.

[80] O. Dammann and A. Leviton, "Intermittent or sustained systemic inflammation and the preterm brain," *Pediatr. Res.*, vol. 75, no. 3, pp. 376–380, Mar. 2014, doi: 10.1038/PR.2013.238.

[81] R. A. Towner *et al.*, "Assessing long-term neuroinflammatory responses to encephalopathy using MRI approaches in a rat endotoxemia model," *Geroscience*, vol. 40, no. 1, pp. 49–60, Feb. 2018, doi: 10.1007/S11357-018-0009-Z.

[82] K. Erikson *et al.*, "Brain tight junction protein expression in sepsis in an autopsy series," *Crit. Care*, vol. 24, no. 1, Jun. 2020, doi: 10.1186/S13054-020-03101-3.

[83] T. Van Der Poll, F. L. Van De Veerdonk, B. P. Scicluna, and M. G. Netea, "The immunopathology of sepsis and potential therapeutic targets," *Nat. Rev. Immunol.*, vol. 17, no. 7, pp. 407–420, Jul. 2017, doi: 10.1038/NRI.2017.36.

[84] O. Handa, J. Stephen, and G. Cepinskas, "Role of endothelial nitric oxide synthase-derived nitric oxide in activation and dysfunction of cerebrovascular endothelial cells during early onsets of sepsis," *Am. J. Physiol. Heart Circ. Physiol.*, vol. 295, no. 4, Oct. 2008, doi: 10.1152/AJPHEART.00476.2008.

[85] H. Ait-Oufella, E. Maury, S. Lehoux, B. Guidet, and G. Offenstadt, "The endothelium: physiological functions and role in microcirculatory failure during severe sepsis," *Intensive Care Med.*, vol. 36, no. 8, pp. 1286–1298, Aug. 2010, doi: 10.1007/S00134-010-1893-6.

[86] H. R. Bright *et al.*, "Neurocognitive Outcomes at 10 Years of Age in Extremely Preterm Newborns with Late-Onset Bacteremia," *J. Pediatr.*, vol. 187, pp. 43-49.e1, Aug. 2017, doi: 10.1016/J.JPEDS.2017.04.045.

[87] A. Leviton *et al.*, “Neonatal systemic inflammation and the risk of low scores on measures of reading and mathematics achievement at age 10 years among children born extremely preterm,” *Int. J. Dev. Neurosci.*, vol. 66, pp. 45–53, May 2018, doi: 10.1016/J.IJDEVNEU.2018.01.001.

[88] E. Sewell, J. Roberts, and S. Mukhopadhyay, “Association of Infection in Neonates and Long-Term Neurodevelopmental Outcome,” *Clin. Perinatol.*, vol. 48, no. 2, pp. 251–261, Jun. 2021, doi: 10.1016/J.CLP.2021.03.001.

[89] S. A. Coggins and K. Glaser, “Updates in Late-Onset Sepsis: Risk Assessment, Therapy, and Outcomes,” *Neoreviews*, vol. 23, no. 11, pp. 738–755, Nov. 2022, doi: 10.1542/NEO.23-10-E738.

[90] G. N. Y. Chan and R. E. Cannon, “Assessment of Ex Vivo Transport Function in Isolated Rodent Brain Capillaries,” *Curr. Protoc. Pharmacol.*, vol. 76, pp. 7.16.1-7.16.16, Mar. 2017, doi: 10.1002/CPPH.21.

[91] E. C. B. Johnson *et al.*, “Large-scale proteomic analysis of Alzheimer’s disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation,” *Nat. Med.*, vol. 26, no. 5, pp. 769–780, May 2020, doi: 10.1038/S41591-020-0815-6,.

[92] S. Abu-Rumeileh *et al.*, “CSF biomarkers of neuroinflammation in distinct forms and subtypes of neurodegenerative dementia,” *Alzheimers Res. Ther.*, vol. 12, no. 1, Dec. 2019, doi: 10.1186/S13195-019-0562-4,.

[93] K. A. McKay, R. Wickström, J. Hillert, and V. D. Karrenbauer, “Cerebrospinal fluid markers in incident pediatric-onset multiple sclerosis: a nationwide study,” *Sci. Rep.*, vol. 11, no. 1, Dec. 2021, doi: 10.1038/S41598-021-97543-6,.

[94] S. A. Hamed, E. A. Hamed, and M. M. Abdella, “Septic encephalopathy: Relationship to serum and cerebrospinal fluid levels of adhesion molecules, lipid peroxides and S-100B protein,” *Neuropediatrics*, vol. 40, no. 2, pp. 66–72, 2009, doi: 10.1055/S-0029-1231054,.

[95] J. F. Ghersi-Egea, E. Saudrais, and N. Strazielle, “Barriers to Drug Distribution into the Perinatal and Postnatal Brain,” *Pharm. Res.*, vol. 35, no. 4, Apr. 2018, doi: 10.1007/s11095-018-2375-8.

[96] E. Candelario-Jalil, Y. Yang, and G. A. Rosenberg, “Diverse roles of matrix metalloproteinases and tissue inhibitors of metalloproteinases in neuroinflammation and cerebral ischemia,” *Neuroscience*, vol. 158, no. 3, pp. 983–994, Feb. 2009, doi: 10.1016/J.NEUROSCIENCE.2008.06.025.

[97] A. Brunse, A. Abbaspour, and P. T. Sangild, “Brain Barrier Disruption and Region-Specific Neuronal Degeneration during Necrotizing Enterocolitis in Preterm Pigs,” *Dev. Neurosci.*, vol. 40, no. 3, pp. 198–208, Oct. 2018, doi: 10.1159/000488979.

[98] J. J. Hill, A. S. Haqqani, and D. B. Stanimirovic, “Proteome of the Luminal Surface of the Blood-Brain Barrier,” *Proteomes*, vol. 9, no. 4, Dec. 2021, doi: 10.3390/PROTEOMES9040045.

[99] S. Blondel *et al.*, “Vascular network expansion, integrity of blood-brain interfaces, and cerebrospinal fluid cytokine concentration during postnatal development in the normal and jaundiced rat,” *Fluids Barriers CNS*, vol. 19, no. 1, Dec. 2022, doi: 10.1186/S12987-022-00332-0.

[100] N. Agarwal, E. S. Lippmann, and E. V. Shusta, “Identification and expression profiling of blood-brain barrier membrane proteins,” *J. Neurochem.*, vol. 112, no. 3, pp. 625–635, Feb. 2010, doi: 10.1111/J.1471-4159.2009.06481.X.

[101] E. A. Andersson, C. Mallard, and C. J. Ek, “Circulating tight-junction proteins are potential biomarkers for blood-brain barrier function in a model of neonatal hypoxic/ischemic brain injury,” *Fluids Barriers CNS*, vol. 18, no. 1, Dec. 2021, doi: 10.1186/S12987-021-00240-9.

[102] Y. Hamada, S. Ogata, T. Masuda, S. Ito, and S. Ohtsuki, “Development of a method for isolating brain capillaries from a single neonatal mouse brain and comparison of proteomic profiles between neonatal and adult brain capillaries,” *Fluids Barriers CNS*, vol. 20, no. 1, Dec. 2023, doi: 10.1186/S12987-023-00449-W.

[103] N. R. Saunders, M. D. Habgood, and K. M. Dziegielewska, “Barrier mechanisms in the brain, II. Immature brain,” *Clin. Exp. Pharmacol. Physiol.*, vol. 26, no. 2, pp. 85–91, 1999, doi: 10.1046/J.1440-1681.1999.02987.X.

[104] T. S. Batth *et al.*, “Protein Aggregation Capture on Microparticles Enables Multipurpose Proteomics Sample Preparation*,” *Molecular & Cellular Proteomics*, vol. 18, no. 5, pp. 1027a–11035, May 2019, doi: 10.1074/mcp.TIR118.001270.

[105] S. Tyanova *et al.*, “The Perseus computational platform for comprehensive analysis of (prote)omics data,” *Nat. Methods*, vol. 13, no. 9, pp. 731–740, Sep. 2016, doi: 10.1038/nmeth.3901.

[106] Y. Perez-Riverol *et al.*, “The PRIDE database at 20 years: 2025 update,” *Nucleic Acids Res.*, vol. 53, no. D1, pp. D543–D553, Jan. 2025, doi: 10.1093/nar/gkae1011.

[107] B. J. Stoll *et al.*, “Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection,” *JAMA*, vol. 292, no. 19, pp. 2357–2365, Nov. 2004, doi: 10.1001/JAMA.292.19.2357.

[108] D. K. Shah *et al.*, "Adverse neurodevelopment in preterm infants with postnatal sepsis or necrotizing enterocolitis is mediated by white matter abnormalities on magnetic resonance imaging at term," *J. Pediatr.*, vol. 153, no. 2, 2008, doi: 10.1016/J.JPEDS.2008.02.033.

[109] K. B. Nelson, J. M. Dambrosia, J. K. Grether, and T. M. Phillips, "Neonatal cytokines and coagulation factors in children with cerebral palsy," *Ann. Neurol.*, vol. 44, no. 4, pp. 665–675, Oct. 1998, doi: 10.1002/ANA.410440413.

[110] D. J. Murphy, P. L. Hope, and A. Johnson, "Neonatal risk factors for cerebral palsy in very preterm babies: case-control study," *BMJ*, vol. 314, no. 7078, pp. 404–408, 1997, doi: 10.1136/BMJ.314.7078.404.

[111] L. J. Schlapbach *et al.*, "Impact of sepsis on neurodevelopmental outcome in a Swiss National Cohort of extremely premature infants," *Pediatrics*, vol. 128, no. 2, Aug. 2011, doi: 10.1542/PEDS.2010-3338.

[112] L.-W. Wang, Y.-C. Lin, S.-T. Wang, T.-F. Yeh, and C.-C. Huang, "Hypoxic/Ischemic and Infectious Events Have Cumulative Effects on the Risk of Cerebral Palsy in Very-Low-Birth-Weight Preterm Infants," *Neonatology*, vol. 106, no. 3, pp. 209–215, 2014, doi: 10.1159/000362782.

[113] A. Bancalari, T. Muñoz, and P. Martínez, "Prolonged intravenous immunoglobulin treatment in very low birth weight infants with late onset sepsis.," *J. Neonatal. Perinatal. Med.*, vol. 13, no. 3, pp. 381–386, 2020, doi: 10.3233/NPM-190259.

[114] L. J. Schlapbach *et al.*, "Impact of Sepsis on Neurodevelopmental Outcome in a Swiss National Cohort of Extremely Premature Infants," *Pediatrics*, vol. 128, no. 2, pp. e348–e357, Aug. 2011, doi: 10.1542/peds.2010-3338.

[115] L. Dayon *et al.*, “Proteomes of Paired Human Cerebrospinal Fluid and Plasma: Relation to Blood-Brain Barrier Permeability in Older Adults,” *J. Proteome Res.*, vol. 18, no. 3, pp. 1162–1174, Mar. 2019, doi: 10.1021/ACS.JPROTEOME.8B00809.

[116] P. Solár, A. Zamani, L. Kubíčková, P. Dubový, and M. Joukal, “Choroid plexus and the blood-cerebrospinal fluid barrier in disease,” *Fluids Barriers CNS*, vol. 17, no. 1, May 2020, doi: 10.1186/s12987-020-00196-2.

[117] C. D. Whelan *et al.*, “Multiplex proteomics identifies novel CSF and plasma biomarkers of early Alzheimer’s disease,” *Acta Neuropathol. Commun.*, vol. 7, no. 1, Nov. 2019, doi: 10.1186/S40478-019-0795-2.

[118] K. Felgenhauer, G. Schliep, and N. Rapic, “Evaluation of the blood-CSF barrier by protein gradients and the humoral immune response within the central nervous system,” *J. Neurol. Sci.*, vol. 30, no. 1, pp. 113–128, 1976, doi: 10.1016/0022-510X(76)90259-8,.

[119] J. Brettschneider, A. Claus, J. Kassubek, and H. Tumani, “Isolated blood-cerebrospinal fluid barrier dysfunction: prevalence and associated diseases,” *J. Neurol.*, vol. 252, no. 9, pp. 1067–1073, Sep. 2005, doi: 10.1007/S00415-005-0817-9.

[120] F. Deisenhammer, F. Sellebjerg, C. E. Teunissen, and H. Tumani, Eds., *Cerebrospinal Fluid in Clinical Neurology*. Cham: Springer International Publishing, 2015. doi: 10.1007/978-3-319-01225-4.

[121] L. Xian, S. Cheng, W. Chen, C. Zhong, Z. Hu, and X. Deng, “Systematic analysis of MASP-1 serves as a novel immune-related biomarker in sepsis and trauma followed by preliminary experimental validation,” *Front. Med. (Lausanne).*, vol. 11, Feb. 2024, doi: 10.3389/fmed.2024.1320811.

[122] J. B. Larsen, M. A. Laursen, C. L. Hvas, K. M. Larsen, S. Thiel, and A.-M. Hvas, “Reduced Mannose-Binding Lectin-Associated Serine Protease (MASP)-1 is Associated with Disturbed Coagulation in Septic Shock,” *Thromb. Haemost.*, vol. 119, no. 06, pp. 952–961, Jun. 2019, doi: 10.1055/s-0039-1685140.

[123] P. Temesvári, C. S. Abrahám, C. P. Speer, J. Kovács, and P. Megyeri, “Escherichia coli 0111 B4 lipopolysaccharide given intracisternally induces blood-brain barrier opening during experimental neonatal meningitis in piglets.,” *Pediatr. Res.*, vol. 34, no. 2, pp. 182–6, Aug. 1993, doi: 10.1203/00006450-199308000-00016.

[124] H. B. Stolp, K. M. Dziegielewska, C. J. Ek, A. M. Potter, and N. R. Saunders, “Long-term changes in blood-brain barrier permeability and white matter following prolonged systemic inflammation in early development in the rat.,” *Eur. J. Neurosci.*, vol. 22, no. 11, pp. 2805–16, Dec. 2005, doi: 10.1111/j.1460-9568.2005.04483.x.

[125] P.-P. Jiang, S.-S. Peng, S. Pankratova, P. Luo, P. Zhou, and Y. Chen, “Proteins Involved in Synaptic Plasticity Are Downregulated in the Cerebrospinal Fluid of Infants With Clinical Sepsis Complicated by Neuroinflammation.,” *Front. Cell. Neurosci.*, vol. 16, p. 887212, 2022, doi: 10.3389/fncel.2022.887212.